

METHODS AND COMPOSITIONS FOR SCREENING OF  
HUMAN BORNA DISEASE VIRUS

Government Support

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Technical Field

15        The present invention relates generally to the  
Borna disease virus (BDV) or BDV-like viruses and  
specifically to compositions and methods useful for  
diagnosing BDV infection, especially in patients with  
neuropsychiatric disorders. More particularly, the  
invention relates to human BDV-derived virus sequences  
and antigens including peptides and recombinant BDV  
fusion proteins, anti-BDV antigen antibodies and  
20        oligonucleotide primers and use thereof in cellular-  
and molecular-based diagnostic methods.

Background of the Invention

25        Evidence indicates that in addition to a genetic  
contribution, environmental determinants also play a  
role in the etiology of psychiatric disorders  
including schizophrenia and depression (Morozov,  
"Advances in Biological Psychiatry, 12, eds., S.  
Mendlewicz and H. Praag, Karger, New York (1983)).

The hypothesis of a viral contribution is suggested by the realization that viruses can induce progressive neurological disorders associated with diverse pathological findings (Morozov, ibid., (1983); Kurstak et al., "Viruses, Immunity, and Mental Disorders", Plenum, New York (1987); ter Meulen, "Seminars in Neuroscience, 3 (1991)).

Borna disease virus (BDV) is a nonsegmented, negative-stranded (NNS) RNA virus (Briese et al., Proc. Natl. Acad. Sci., USA, 91:4362-4366 (1994); Cubitt et al., J. Virol., 68:1382-1396 (1994); de la Torre, J. Virol., 68:7669-7675 (1994); and Schneemann et al., Virol., 210:1-8 (1995)) with a nuclear site for the replication and transcription of its genome (de la Torre, supra, (1994); Schneemann et al., supra, (1995); and Cubitt et al., J. Virol., 68:1371-1381 (1994)) and the use of RNA splicing for its gene expression regulation (Cubitt et al., Virus Res., 34:69-79 (1994) and Schneider et al., J. Virol., 68:5007-5012 (1994)). These features signal BDV as the prototype of a new group of animal viruses (de la Torre, supra, (1994) and Schneemann et al., supra, (1995)).

Borna disease virus (BDV) is a noncytolytic neurotropic virus that infects a wide range of vertebrate species from birds and rodents to primates. It has a variable period of incubation and diverse pathological manifestations depending on the species, immune status and age of the host, as well as route of

infection and virus strain (Ludwig et al., Prog. Med. Virol., 35:107-151 (1988); Lipkin et al., Microbial Pathogenesis, 13:167-170 (1992); Richt et al., Clin. Infect. Dis., 14:1240-1250 (1992); Koprowski et al.,  
5 Curr. Topics Microbiol. Immunol., 190 (1995)).

Thus, BDV causes CNS disease in several non-human vertebrate species that is manifested by behavioral abnormalities and diverse pathologies depending on the species, age and immune status of the host, as well as  
10 route of infection and virus strain (Rott et al., in "Borna Disease", eds., H. Koprowski and I. Lipkin, Springer-Verlag, Berlin, pp17-30 (1995)). For example, heightened viral expression in limbic system structures, together with astrocytosis and neuronal  
15 degeneration within the hippocampal formation, constitute the main histopathological hallmarks of BDV infection in different animal species (Goszttonji et al., in "Borna Disease", eds., H. Koprowski and I. Lipkin, Springer-Verlag, Berlin, pp39-73 (1995) and  
20 Carbone et al., J. Virol., 65:6154-6164 (1991)).

In the recently published International Application WO 96/21020, rat-derived BDV viral sequences were described for encoding rat BDV polypeptide sequences corresponding to p40, p23, gp18,  
25 p57, and BDV polymerase sequences. In addition, the application presents diagnostic and therapeutic methods for treating nervous system diseases based on the use of the rat-derived nucleic acids and encoded

polypeptides. However, no human-specific sequences were identified by the authors.

The reproducible and clinically definable behavioral abnormalities accompanying BDV infection of rats and non-human primates have led to the speculation that BDV could cause similar CNS dysfunctions in humans. In support of this hypothesis are the results from cross-sectional seroepidemiological studies showing an increased prevalence of antibodies that recognize BDV-specific antigens in subjects with neuropsychiatric disorders compared to the normal healthy population (Rott et al., Science, 228:755-756 (1985); Bode et al., Lancet, ii:689 (1988); VandeWoude et al., Science, 250:1278-1281 (1990); Rott et al., Arch. Virol., 118:143-149 (1991); Bode et al., J. Med. Virol., 36:309-315 (1992); Fu et al., J. Affect. Disor., 27:61-68 (1993), for review see Bode, Curr. Top. Microbiol. Immunol., 190:101-128 (1995)). Moreover, prospective studies on acute psychiatric patients have shown a high percentage of BDV seropositives among patients with major depression (Bode et al., Arch. Virol. (Suppl), 7:159-167 (1993); Bode et al., Lancet, 343:297-298 (1994); and Bode et al., Nature Med., 1:232-236 (1995)).

Recently, using flow cytometry (FCM), BDV-specific antigens have been detected in peripheral blood monocytes (PBMC) from psychiatric patients (Bode et al., supra, (1994)). In addition, the present

inventors with others have detected BDV-specific RNA sequences in such PBMC (Bode et al., supra, (1995) and Kishi et al., FEBS Letters, 364:293-297 (1995)).

These findings led the present inventors to

5 investigate the possibility of isolating infectious BDV from BDV-antigen positive human PBMC.

The present invention describes the isolation and sequence characterization of human BDV. Studies using coded PBMC samples from psychiatric patients and  
10 healthy control subjects for co-cultivation with a human oligodendroglia cell line (OL cells), led to the isolation of BDV from three hospitalized psychiatric patients, but not from any of the control subjects.

The isolated virus was unequivocally identified as BDV  
15 based on the sequence identification of BDV open reading frames (ORFs) p24, p16, p56, and the putative catalytic domain of the BDV L polymerase. The sequence analysis obtained with the methods and compositions of this invention indicate that BDV human  
20 isolates are genetically very closely related to BDV from naturally infected animals of different species. These results further indicate that BDV could be one of the environmental factors contributing to the pathophysiology of neuropsychiatric disorders whose  
25 etiology remains elusive.

The present invention describes the detection of novel BDV antigen and RNA in the CNS of patients who presented with a history of mental disorders.

BDV-specific antigen and RNA was also determined for

the first time for the p16, p56 and L polymerase BDV-encoded polypeptides.

Thus, the present invention now unequivocally identifies the presence of infectious BDV in humans and its association with clinical profiles of mental disorders whose etiology remains unknown.

#### Brief Summary of the Invention

The present invention therefore relates to methods, diagnostic systems and compositions useful for detecting human BDV or human BDV-like viral infection in a subject.

Compositions for use in the present invention include human BDV nucleic acids, vectors containing the nucleic acids, cells containing the vectors, human BDV polypeptides encoded by the nucleic acids or derived from a partial amino acid sequence therefrom, and anti-BDV polypeptide antibodies.

Preferred BDV nucleic acids encode a human BDV p24 polypeptide comprising an amino acid residue sequence in SEQ ID NOS 20, 21, 22, 32 and 33. Preferred p24 encoding nucleic acids have the nucleotide sequence in SEQ ID NOS 3, 4 and 5.

Other preferred BDV nucleic acids encode a human p16 polypeptide comprising an amino acid residue sequence in SEQ ID NOS 23, 24, 25, 34 and 35. Preferred p16 encoding nucleic acids have the nucleotide sequence in SEQ ID NOS 7, 8 and 9.

Still other preferred BDV nucleic acids encode a human p56 polypeptide comprising an amino acid residue sequence in SEQ ID NOs 26, 27, 36, 37 and SEQ ID NO 38. Preferred p56 encoding nucleic acids have the nucleotide sequence in SEQ ID NOs 11 and 12.

Further preferred BDV nucleic acids encode a human BDV p40 polypeptide with the amino acid residue sequence in SEQ ID NOs 28, 29, 30, 39, 40 and 41. Preferred p40 encoding nucleic acids have the nucleotide sequence in SEQ ID NOs 14, 15 and 16.

Other preferred BDV nucleic acids encode a human BDV catalytic domain polypeptide of L polymerase protein with the amino acid residue sequence in SEQ ID NO 31. Preferred catalytic domain encoding nucleic acids have the nucleotide sequence in SEQ ID NOs 18 and 19.

Expression vectors containing the above identified BDV nucleic acids are also contemplated and in preferred aspects, the BDV nucleic acid is operably linked to a promoter. Cells transformed with the above identified expression vectors are contemplated.

The preferred BDV p24, p16, p56, p40 and catalytic domain polypeptides are identified above where the polypeptides are either synthetic or recombinant. Fusion proteins are also contemplated.

Antibodies that immunoreact with human BDV and the human BDV polypeptides of this invention are further contemplated.

Methods for use in the present invention include nucleic acid based as well as protein based methods for respectively detecting BDV nucleic acids and proteins. For the former, the method involves  
5 hybridizing a nucleic acid in a sample with a BDV nucleic acid of this invention. In preferred embodiments, the sample is a BDV-infectable cell, preferably a peripheral blood mononuclear cell. The sample is preferably isolated from a human and is  
10 useful for diagnosing BDV infection. In preferred embodiments, the method is useful to diagnose infection in a subject having a neuropsychiatric disorder.

Protein based methods include detection of a  
15 BDV ligand in a sample by contacting the sample with a human BDV polypeptide described above to allow formation of an immunoreaction complex followed by detection thereof. Preferred BDV ligands are antibodies. In preferred aspects, detection is  
20 accomplished with the addition of a detecting antibody that binds to the immunoreaction complex or by the indirect immunofluorescence focus assay. Detecting antibodies may also be labeled. In other preferred aspects, the polypeptide is immobilized on a solid  
25 support. Samples include a body fluid, preferably serum. The method is particularly useful for diagnosing BDV infection in a human.



The method according to claim 66 wherein the sample is isolated from a human having a neuropsychiatric disorder.

Other preferred methods include detecting a BDV antigen in a sample by contacting the sample with an anti-human BDV antibody as described above thereby forming an immunoreaction complex followed by detection thereof. In preferred aspects, the sample comprises cells, most preferably peripheral blood mononuclear cells. Detection can be accomplished by flow cytometry, ELISA, or by immunoblot analysis.

The present invention also contemplates kits for detecting the presence of BDV nucleic acid, BDV ligands or BDV antigens as described above.

#### Brief Description of the Drawings

Figure 1 is a schematic diagram of the BDV genome RNA (anti-genomic polarity) and location of primers used in amplifying the genome encoding BDV p40, p24, p16, p56 and a portion of the L polymerase polypeptides. The genome organization presented in the diagram is based on the complete sequence of two non-human BDV RNA genomes as yet reported, strain V and C6BV (Briese et al., Proc. Natl. Acad. Sci. USA, 91:4382-4386 (1994); Cubitt et al., J. Virol., 68:1382-1396 (1994); and de la Torre, J. Virol., 68:7669-7675 (1994)). Accession numbers for strain V and C6BV RNA genome sequences are U04608 and L27077, respectively. Sequence, polarity, and nucleotide

positions covered by the primers are summarized in Table 1 and Table 2.

Figures 2A-2H illustrate the continuous cDNA nucleotide sequence alignment of the BDV strains described by de la Torre, J. Virol., 68:7669-7675 (1994) indicated as BDV JCT and by Brieese et al., Proc. Natl. Acad. Sci. USA, 91:4382-4386 (1994) indicated as BDV Brieese. Dots indicate the same nucleotide sequence as that found in the same position in the consensus sequence. Nucleotide substitutions for each strain are indicated above the consensus sequence. At those positions, the consensus sequence presents the possible substitutions according to the convention adopted by the IUPAC-IUB Biochemical Nomenclature Commission. The consensus sequence is also listed as SEQ ID NO 1.

Figures 3A and 3B together illustrate the expression of human BDV RNA in PBMC of psychiatric patients. Total RNA (1 to 5  $\mu$ g) isolated from PBMC was reverse transcribed by priming with random hexamers and the corresponding cDNAs amplified by PCR as described in Examples 1 and 2 using the following:

- 1) BDV-specific primers to amplify a 603 bp fragment corresponding to full length BDV p24 ORF including 15 other bp of primer sequences outside the ORF. BDV specificity was determined by southern blot hybridization as shown in Figure 2A using a BDV-specific probe internal to the predicted PCR product;
- 2) Specific primers to amplify a 192 bp

GAPDH fragment detected by ethidium bromide staining as indicated in Figure 2B. Samples are: lane 1, PBMC from a representative healthy control individual negative for BDV antigen; lanes 2-4, PBMC from  
5 psychiatric patients H1, H2, and H3, respectively. Figures 3A and 3B together form a composite of the autoradiographic segment showing the results of southern blot hybridization and the part of the gel showing the ethidium bromide staining of GAPDH  
10 amplified fragment. Track M corresponds to the 1 kb ladder DNA. The top and bottom parts of the composite were lined up with respect to the migration of the 1 kb ladder DNA (track M).

Figures 4A, 4B and 4C separately illustrate the  
15 respective nucleotide sequence alignment of open reading frames (ORFs) p24 (4A), p16 (4B) and p56 (4C) among the human BDV isolates (H1, H2, and H3), C6BV and BDV strain V. Dots indicate the same nucleotide as the one found for that position in the BDV strain V  
20 sequence. Numbers on the right correspond to last nucleotide position of each row within the corresponding ORF. In Figure 4A, the nucleotide sequence for Strain V is listed as SEQ ID NO 2 while the nucleotide sequences for p24 for each of patients  
25 H1, H2 and H3 are respectively listed as SEQ ID NOs 3, 4 and 5. In Figure 4B, the nucleotide sequence for Strain V is listed as SEQ ID NO 6 while the nucleotide sequences for p16 for each of patients H1, H2 and H3 are respectively listed as SEQ ID NOs 7, 8 and 9. In

Figure 4C, the nucleotide sequence for Strain V is listed as SEQ ID NO 10 while the nucleotide sequence for p56 for patient H1 is listed as SEQ ID NO 11. Since the nucleotide sequences for patients H2 and H3 are identical, that sequence is listed as SEQ ID NO 12.

Figures 5A-1 and 5A-2 illustrate the nucleotide sequence alignment of the p40 open reading frame (ORF) among the human BDV isolates (H1, H2, and H3), C6BV and BDV strain V along with a derived consensus sequence written using the IUPAC code. Dots indicate the same nucleotide as the one found for that position in the consensus sequence (SEQ ID NO 13). Numbers on the right correspond to last nucleotide position of each row within the corresponding ORF. In Figures 5A-1 and 5A-2, the nucleotide sequences for each of patients H1, H2 and H3 are respectively listed as SEQ ID NOS 14, 15 and 16.

Figures 5B-1, 5B-2 and 5B-3 illustrate the nucleotide sequence alignment of the open reading frames (ORF) of the catalytic domain of L polymerase, labeled on the figures as p180, among the human BDV isolates (H1, H2, and H3), C6BV (labeled as p180frag) and BDV strain V (5) along with a derived consensus sequence written using the IUPAC code (SEQ ID NO 17). Dots indicate the same nucleotide as the one found for that position in the consensus sequence. Numbers on the right correspond to last nucleotide position of each row within the corresponding ORF. Since the

nucleotide sequences for patients H1 and H2 are identical, that sequence is listed as SEQ ID NO 18 while that for patient H3 is listed as SEQ ID NO 19.

Figure 6A shows the amino acid differences found in ORFs p24, p16 and p56 among the human BDV isolates (H1, H2, and H3), C6BV and strain V. Amino acids are presented in the single letter code. Numbers on top correspond to the codon position within each ORF. Figure 6B is a triangular matrix summarizing the total number of nucleotide (upper right) and amino acid (lower left) substitutions among human BDV isolates (H1, H2, and H3), C6BV and strain V.

Figure 7A illustrates the expression of BDV RNA in OL cells infected by co-cultivation with PBMC from psychiatric patients. Total RNA (10  $\mu$ g) from each sample was analyzed by Northern blot hybridization using specific probes for BDV p24 and GAPDH, respectively shown in Figure 7A and Figure 7B. Lanes 1 and 5 correspond to RNA from OL cells infected with PBMC from two representative healthy control individuals negative for BDV antigen; lanes 2-4 correspond to RNA from OL cells infected with PBMC from patients H1, H2, and H3, respectively. RNA from C6 (lane 6) and C6BV (lane 7) cells, were used as negative and positive controls, respectively, of BDV hybridization. Figure 7C shows the ethidium bromide staining of the RNA gel.

Figures 8A, 8B and 8C respectively show the p24 amino acid residue sequences, also listed as SEQ ID

NOs 20, 21 and 22, derived from patient nucleotide sequences H1, H2 and H3 listed in SEQ ID NOs 3, 4 and 5 as described in the legend for Figure 4A.

Figures 9A, 9B and 9C respectively show the p16 amino acid residue sequences, also listed as SEQ ID NOs 23, 24 and 25, derived from patient nucleotide sequences H1, H2 and H3 listed in SEQ ID NOs 7, 8 and 9 as described in the legend for Figure 4B.

Figures 10A and 10B respectively show the p56 amino acid residue sequences, also listed as SEQ ID NOs 26 and 27, derived from patient nucleotide sequences H1 listed as SEQ ID NO 11 and H2/H3 (identical) listed in SEQ ID NO 12 as described in the legend for Figure 4C.

Figures 11A, 11B and 11C respectively show the p40 amino acid residue sequences, also listed as SEQ ID NOs 28, 29 and 30, derived from patient nucleotide sequences H1, H2 and H3 listed in SEQ ID NOs 14, 15 and 16 as described in the legend for Figure 5A.

Figure 12 shows the amino acid residue sequence of the catalytic domain of the L polymerase, also listed as SEQ ID NO 31, derived from patient nucleotide sequences H1/H2 (identical) and H3 respectively listed in SEQ ID NOs 18 and 19 as described in the legend for Figure 5B.

Detailed Description of the Invention

A. Definitions

TABLE OF CORRESPONDENCE

	<u>Code Group</u>		<u>Nucleotide(s)</u>
5	A	A	adenine
	C	C	cytosine
	G	G	guanine
	T	T	thymine (in DNA)
	U	U	uracil (in RNA)
10	Y	C or T(U)	pyrimidine
	R	A or G	purine
	M	A or C	amino
	K	G or T(U)	keto
	S	G or C	strong interaction (3 hydrogen bonds)
15	W	A or T(U)	weak interaction (2 hydrogen bonds)
	H	A or C or T(U)	not-G
	B	G or T(U) or C	not-A
	V	G or C or A	not-T or not-U
	D	G or A or T(U)	not-C
20	N	G,A,C or T(U)	any

Nucleotide: A monomeric unit of DNA or RNA consisting of a sugar moiety (pentose), a phosphate, and a nitrogenous heterocyclic base. The base is  
 25 linked to the sugar moiety via the glycosidic carbon (1' carbon of the pentose) and that combination of base and sugar is a nucleoside. When the nucleoside contains a phosphate group bonded to the 3' or 5' position of the pentose it is referred to as a

nucleotide. A sequence of operatively linked nucleotides is typically referred to herein as a "base sequence" or "nucleotide sequence", and their grammatical equivalents, and is represented herein by a sequence whose left to right orientation is in the conventional direction of 5'-terminus to 3'-terminus.

Base Pair (bp): A partnership of adenine (A) with thymine (T), or of cytosine (C) with guanine (G) in a double stranded DNA molecule. In RNA, uracil (U) is substituted for thymine.

Nucleic Acid: A polymer of nucleotides, either single or double stranded. When referring to nucleic acids, the term "substantial identity" indicates that the sequences of two nucleic acids, or designated portions thereof, when optimally aligned and compared, are identical, with appropriate nucleotide insertions or deletions, in at least about 80% of the nucleotides, usually at least 90% to 95%, and more preferably at least about 98% to 99.5% of the nucleotides. Alternatively, substantial nucleic acid identity exists when a nucleic acid segment will hybridize under selective hybridization conditions, to a complement of another nucleic acid strand.

Polynucleotide: A polymer of single or double stranded nucleotides. As used herein "polynucleotide" and its grammatical equivalents will include the full range of nucleic acids. A polynucleotide will typically refer to a nucleic acid molecule comprised of a linear strand of two or more deoxyribonucleotides



and/or ribonucleotides. The exact size will depend on many factors, which in turn depends on the ultimate conditions of use, as is well known in the art. The polynucleotides of the present invention include  
5 primers, probes, RNA/DNA segments, oligonucleotides or "oligos" (relatively short polynucleotides), genes, vectors, plasmids, and the like.

Gene: A nucleic acid whose nucleotide sequence codes for a polypeptide. The primary information can  
10 either be RNA or DNA.

Duplex DNA: A double-stranded nucleic acid molecule comprising two strands of substantially complementary polynucleotides held together by one or more hydrogen bonds between each of the complementary  
15 bases present in a base pair of the duplex. Because the nucleotides that form a base pair can be either a ribonucleotide base or a deoxyribonucleotide base, the phrase "duplex DNA" refers to either a DNA-DNA duplex comprising two DNA strands (ds DNA), or an RNA-DNA  
20 duplex comprising one DNA and one RNA strand.

Complementary Bases: Nucleotides that normally pair up when DNA or RNA adopts a double stranded configuration.

Complementary Nucleotide Sequence: A sequence of  
25 nucleotides in a single-stranded molecule of DNA or RNA that is sufficiently or substantially complementary to that on another single strand to specifically and selectively hybridize to it with consequent hydrogen bonding. Selectivity of

hybridization exists when hybridization occurs that is more selective than total lack of specificity.

Typically, selective hybridization will occur when there is at least about 55% identity over a stretch of  
5 at least 14-25 nucleotides, preferably at least about 65%, more preferably at least about 75%, and most preferably at least about 90%.

Hybridization: The pairing of substantially complementary nucleotide sequences (strands of nucleic  
10 acid) to form a duplex or heteroduplex by the establishment of hydrogen bonds between complementary base pairs. It is a specific, i.e., non-random, interaction between two complementary polynucleotides that can be competitively inhibited.

Isolated or Substantially Purified: With nucleic  
15 acids, the terms refer to those that have been purified away from other cellular components or other contaminants, e.g., other cellular nucleic acids or proteins, by standard techniques, including  
20 alkaline/SDS treatment, CsCl banding, column chromatography, and others well known in the art.

Nucleotide Analog: A purine or pyrimidine nucleotide that differs structurally from A, T, G, C, or U, but is sufficiently similar to substitute for  
25 the normal nucleotide in a nucleic acid molecule.

Upstream: In the direction opposite to the direction of DNA transcription, and therefore going from 5' to 3' on the noncoding strand, or 3' to 5' on the RNA transcript.

Downstream: Further along a DNA sequence in the direction of sequence transcription or read out, that is, traveling in a 3'- to 5'-direction along the noncoding strand of the DNA or 5'- to 3'-direction along the RNA transcript.

Stop Codon: Any of three codons that do not code for an amino acid, but instead cause termination of protein synthesis. They are UAG, UAA and UGA and are also referred to as a nonsense, termination, or translational stop codon.

Reading Frame: Particular sequence of contiguous nucleotide triplets (codons) employed in translation. The reading frame depends on the location of the translation initiation codon.

Amino Acid Residue: An amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are preferably in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property is retained by the polypeptide. NH<sub>2</sub> refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In keeping with standard polypeptide nomenclature (described in J. Biol. Chem., 243:3552-59 (1969) and adopted at 37 CFR §1.822(b)(2)), abbreviations for amino acid residues are shown in the following Table of Correspondence:

TABLE OF CORRESPONDENCE

		<u>SYMBOL</u>		<u>AMINO ACID</u>
		<u>1-Letter</u>	<u>3-Letter</u>	
		Y	Tyr	tyrosine
5		G	Gly	glycine
		F	Phe	phenylalanine
		M	Met	methionine
		A	Ala	alanine
		S	Ser	serine
10		I	Ile	isoleucine
		L	Leu	leucine
		T	Thr	threonine
		V	Val	valine
		P	Pro	proline
15		K	Lys	lysine
		H	His	histidine
		Q	Gln	glutamine
		E	Glu	glutamic acid
		Z	Glx	Glu and/or Gln
20		W	Trp	tryptophan
		R	Arg	arginine
		D	Asp	aspartic acid
		N	Asn	asparagine
		B	Asx	Asn and/or Asp
25		C	Cys	cysteine
		X	Xaa	Unknown or other

It should be noted that all amino acid residue sequences are represented herein by formulae whose

left and right orientation is in the conventional direction of amino-terminus to carboxy-terminus.

Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino acid residues.

Polypeptide: A linear series of amino acid residues connected to one another by peptide bonds between the alpha-amino group and carboxy group of contiguous amino acid residues.

Protein: A linear series of greater than 50 amino acid residues connected one to the other as in a polypeptide.

Substantially Purified or Isolated: When used in the context of polypeptides or proteins, the terms describe those molecules that have been separated from components that naturally accompany them. Typically, a monomeric protein is substantially pure when at least about 60% to 75% of a sample exhibits a single polypeptide backbone. Minor variants or chemical modifications typically share the same polypeptide sequence. A substantially purified protein will typically comprise over about 85% to 90% of a protein sample, more usually about 95%, and preferably will be over about 99% pure. Protein or polypeptide purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a sample, followed by visualization thereof by staining. For certain purposes, high

resolution is needed and high performance liquid chromatography (HPLC) or a similar means for purification utilized.

Synthetic Peptide: A chemically produced chain of amino acid residues linked together by peptide bonds that is free of naturally occurring proteins and fragments thereof.

B. BDV and Infection in Humans

Borna disease virus (hereinafter referred to as BDV) is a noncytolytic neurotropic virus that has been shown to infect a wide range of vertebrate species from birds and rodents to primates. BDV is a nonsegmented, negative-stranded (NNS) RNA enveloped virus, that, as described in the Background, is the prototype of a new group of animal RNA viruses. As used herein, the term "BDV" is used in conjunction with viral particles, nucleic acids, polypeptides and antibodies.

In addition to a genetic contribution, environmental determinants including viral infection play a role in the etiology of psychiatric disorders including schizophrenia and depression. Recent evidence shows that patients with psychiatric disorders contain protein and nucleic acid markers of the presence of BDV. Specifically, the p24 and p38/40 BDV-specific antigens (Bode et al., Lancet, 343:297-298 (1994) and corresponding RNA sequences (Bode et al., Nature Med., 1:232-236 (1995) and Kishi et al.,

FEBS Letters, 364:293-297 (1995) have been detected in CD14<sup>+</sup> peripheral blood monocytes (PBM) subset within peripheral blood mononuclear cells (PBMC) from patients with psychiatric disorders.

5        However, the isolation of infectious BDV from BDV antigen-positive PBMC was not accomplished prior to the present invention. In addition, this invention presents the first documentation of BDV RNA in a human that encodes full length 24 polypeptide (p24) as well  
10       as p16 and p56 polypeptides. Furthermore, the presence of a catalytically active portion of the BDV L polymerase has now been documented in human patients.

15       Thus, for the first time, the isolation and sequence characterization of human BDV is now described. As described in the Examples, from coded PBMC samples from psychiatric patients and healthy control subjects for co-cultivation with a human oligodendroglia cell line (OL cells), BDV was isolated  
20       from three hospitalized psychiatric patients, but not from any of the control subjects. The isolated virus was unequivocally identified as BDV based on the sequence identification of BDV open reading frames (ORFs) p24, p16, p56, and the putative catalytic  
25       domain of the BDV L polymerase. Furthermore, the identification of infectious human BDV was confirmed by the infection of a human oligodendroglial cell line following co-cultivation with BDV-positive patient PBMC as described in the Examples. Therefore, the

viral isolates have been unequivocally identified as infectious human BDV.

The present invention thus contemplates an isolated infectious human BDV. A human BDV isolate is  
5 a nonsegmented, negative-stranded (NNS) RNA enveloped virus that is present in humans, specifically in cells or body fluids of a human as discussed below. The human BDV particle is characterized by containing an RNA genome also referred to as genomic viral nucleic  
10 acid. BDV isolated from different subjects suspected of having BDV, although related, may display differences in nucleotide sequence that may or may not result in encoded amino acid differences in the translated BDV polypeptides.

15 Thus, a BDV genomic viral nucleic acid within a BDV isolate is specific for the subject from which it is obtained. In other words, the BDV nucleic acid is said to be "derived from" a specific BDV-infected cell obtained from a particular subject. In particular, as  
20 described in Section C below, the BDV nucleic acid sequences derived from three BDV-infected patients exhibited nucleotide substitutions at various positions within the genome thereby rendering each sequence unique. However, some polypeptide-encoding  
25 regions of the genome from the three patients were identical. As discussed later, for the regions that did reveal nucleotide substitutions, some of them resulted in changes in the deduced amino acid sequence of various BDV polypeptides.



As used herein, the terms "isolated",  
"substantially isolated", "substantially purified" and  
"substantially homogenous" are used interchangeably  
and describe a molecule that has been separated from  
5 components that naturally accompany it. When  
referring to a BDV isolate, "isolated" refers to those  
BDV isolates that have been purified away from other  
cellular or fluid components, e.g., other cellular  
nucleic acids, including other viruses, or proteins  
10 found within cells or in a body fluid sample. Thus, a  
human BDV isolate represents BDV particles that have  
been separated from cellular components or fluids in  
which the particle resides. Exemplary techniques for  
isolating BDV particles of this invention are well  
15 known in the art.

A BDV particle-containing cell is also referred  
to as a BDV-infected cell or a BDV-containing cell.  
In other words, a BDV-containing cell of this  
invention is a cell that is infected with a human BDV  
20 particle as defined herein. With respect to BDV  
particles within cells, one aspect of the present  
invention includes isolation of BDV particles from  
cells derived directly from a person. Such cells are  
obtained from a person suspected of having BDV. A  
25 preferred BDV-infected cell in a subject is a  
peripheral blood mononuclear cell also referred to as  
PBMC from which BDV is isolatable. Such cells are  
obtained by standard techniques, e.g., purification

from a blood sample, leukophoresis, and others well known in the art.

Another aspect of the present invention includes isolation of BDV particles from cultured cells that have been infected with BDV by co-cultivation or exposure to BDV-infected cells derived from a BDV-infected person. A preferred BDV-infectable cell line is a human oligodendroglial cell line. Other BDV-infectable cell lines contemplated for use in isolating patient-specific BDV include human neuroblastoma cell lines.

In view of the ability to isolate BDV from BDV-infected PBMC following co-cultivation with uninfected oligodendroglial cells, the isolated virus is said to be infectious, i.e., have the ability to infect and multiply in susceptible cells. Thus, the term "infectious" describes a process whereby BDV is capable of being spread from one cell to another, with or without direct contact. The process can therefore occur both in vivo and in vitro. As a result, BDV is said to invade and become established in or on the recipient or infected cell. Such infection may be active whereby the BDV multiplies within the cell. This process may result in the state of local or systemic disease with cellular or systemic injury if the cell is within an organism. Alternatively, the infectious BDV may be present at very low levels in the infected subject and, in this case BDV may not be recovered from such a subject although its continued

presence can be inferred from continuing immunologic reactivity, or retrospectively, from the later emergence of overt illness.

In another aspect of the present invention, BDV is isolated from a sample taken from a subject suspected of having BDV. As used herein, a "sample", also referred to as a body sample or a fluid sample, is any sample that can be removed from a subject and in which BDV resides. Exemplary samples include serum and PBMC. Methods of collecting such samples are well known in the medical arts, such as withdrawal of blood samples by venipuncture.

The human BDV particles of this invention are found in subjects who have been diagnosed and characterized as having psychiatric disorders as described in the Examples. Based on the present discoveries, BDV has been found in patients diagnosed with acute bipolar disorders with or without psychotic features and in patients diagnosed with chronic obsessive compulsive disorder. Thus, patients exhibiting behavioral characteristics known to be associated with these psychiatric profiles are potential candidates for screening for BDV infection and isolating BDV therefrom. The phrase "suspected of having BDV" describes subjects who are characterized by having the above-described psychiatric profiles as well as the characteristics of other neuropsychiatric disorders including schizophrenia.

Thus, isolation of infectious human BDV particles in psychiatric patients has provided support for a viral-mediated etiology of some forms of neurological dysfunction.

5  
C. Human BDV Nucleic Acids

The nucleic acids of the present invention are BDV nucleic acids that are isolatable from a human. As such, BDV nucleic acids include complete  
10 genomic RNA nucleic acid sequences, DNA sequences complementary to BDV genomic RNA sequences, RNA and the corresponding cDNA sequence regions that encode BDV polypeptides, complementary sequences thereto, and fragments thereof, such as shorter polynucleotide  
15 sequences useful for hybridization aspects of this invention. The term "BDV nucleic acid" thus includes RNA genomic and cDNA sequences of the BDV genome. The nucleic acids of this invention further include sequences having other nucleotides known in the art  
20 such as nucleotide analogs.

The nucleic acids of this invention may be present in infected cells, in an infected or transfected cell lysate, in body fluid samples, in partially purified form, or in substantially pure  
25 form. As an alternative from isolating BDV nucleic acids from natural sources as described herein, BDV nucleic acids may be synthetic molecules. The nucleic acid can be in the form of nucleic acids described above, such as genomic RNA or cDNA, and in single or

double stranded form, respectively. The terms "nucleic acid" and "polynucleotide" been previously defined in Section A.

5 Nucleic acids that correspond to the entire BDV viral genome or portions thereof, that are able to cause BDV infection when transfected into susceptible cells, or that code for the claimed BDV polypeptides or portions thereof, will normally be from at least 10 to thousands of nucleotide base pairs in length. One  
10 or more introns may be present in the genome including the protein-coding nucleic acid sequences. The length of nucleic acid sequences will vary depending on the use. In view of the degeneracy of the genetic code for encoding amino acids, different nucleotide  
15 sequences including encoding triplet codons are contemplated in this invention that encode substantially the same or functionally the equivalent amino acid sequence of the BDV polypeptides as described herein and below.

20 BDV nucleic acids contain open reading frames (ORF) that encode BDV polypeptides, and fragments thereof, include those that encode all or part of the BDV polypeptides designated p24, p16, p56, p40 and the L polymerase, preferably the catalytic domain. The  
25 polypeptides are more fully described in Section D.

Thus, a preferred nucleic acid encodes a human BDV p24 polypeptide having an amino acid residue sequence as listed in SEQ ID NO 20, SEQ ID NO 21, SEQ ID NO 22, MATGPSSLVDSLEDEEDP (SEQ ID NO 32) and

RIYPQLPSAPTADEWDIIP (SEQ ID NO 33). Similarly, a preferred nucleic acid encodes a human BDV p16 polypeptide having an amino acid residue sequence as listed in SEQ ID NO 23, SEQ ID NO 24, SEQ ID NO 25, 5 MNSKHSYVELKGKVIVPG (SEQ ID NO 34) and RLRNIGVGPLGPDIRSSGP (SEQ ID NO 35). Another preferred nucleic acid encodes a human BDV p56 polypeptide having an amino acid residue sequence as listed in SEQ ID NO 26, SEQ ID NO 27, GLSCNTDSTPGLIDLEIR (SEQ ID NO 10 36), RSKLRRRRRDTQQIEYLV (SEQ ID NO 37) and LISLCVSLPASFARRRRLGRWQE (SEQ ID NO 38). A further preferred nucleic acid encodes a human BDV p40 polypeptide having an amino acid residue sequence as listed in SEQ ID NO 28, SEQ ID NO 29, SEQ ID NO 30, 15 MPPKRRLVDDADAMEDQD (SEQ ID NO 39), MEDQDDLYEPPSSLPKLP (SEQ ID NO 40) and ELSGEISAIMRMIGLTGLN (SEQ ID NO 41). A further preferred nucleic acid encodes a human BDV catalytic domain polypeptide from L polymerase having an amino acid residue sequence as listed in SEQ ID NO 20 31.

While all nucleic acid sequences are contemplated for encoding the BDV polypeptides as described above based on the degeneracy of the genetic code, particularly preferred p24 BDV-polypeptide encoding 25 nucleic acid sequences are those listed in SEQ ID NO 3, SEQ ID NO 4 and SEQ ID NO 5. Similarly, particular p16 BDV-polypeptide encoding nucleic acid sequences are those listed in SEQ ID NO 7, SEQ ID NO 8 and SEQ ID NO 9. Particular p56 BDV-polypeptide encoding

nucleic acid sequences are those listed in SEQ ID NO 11 and SEQ ID NO 12. BDV p40 polypeptide encoding nucleic acid sequences are listed in SEQ ID NO 14, SEQ ID NO 15 and SEQ ID NO 16. BDV catalytic domain polypeptide encoding nucleic acid sequences are listed in SEQ ID NO 18 and SEQ ID NO 19.

Polynucleotide sequences derived from the above sequences are also contemplated in the present invention. Such sequences are useful as hybridization probes for the presence of BDV nucleic acids in physiological body samples including cells and body fluids as well as in laboratory samples such as in DNA libraries including genomic and cDNA libraries, tissue extracts, cell extracts, and in impure and purified samples and the like. Other sequences are useful as primers for use in PCR amplification of BDV sequences as more fully described in Section F1. For either aspect the polynucleotide sequences will usually be at least about 10 nucleotides in length and more usually 15 to 25 nucleotides in length.

These polynucleotide sequences may be synthetic DNA fragments prepared, for example, by the phosphoramidite method described by Beaucage et al., Tetrahedron Letters, 22:1859-1862 (1981), or by the triester method according to Matteucci et al., J. Am. Chem. Soc., 103:3185 (1981), both incorporated herein by reference. See also, U.S. Patents No. 4,356,270, No. 4,458,066, No. 4,416,988, No. 4,293,652. A double stranded fragment may then be obtained, if desired, by

annealing the chemically synthesized single strands together under appropriate conditions or by synthesizing the complementary strand using DNA polymerase with an appropriate primer sequence.

5        A polynucleotide of this invention is substantially complementary to a target BDV nucleic acid when it will anneal only to a single desired position on that target nucleic acid under conditions as described in the Examples. Proper annealing  
10       conditions depend, for example, upon the polynucleotide's length, base composition, and the number of mismatches and their position on a polynucleotide, and must often be determined empirically.

15       The primer is capable of acting as a point of initiation of nucleic acid synthesis when placed under conditions in which synthesis of a primer extension product, complementary to a nucleic acid strand, is induced. Inducing conditions include the presence of  
20       nucleotides and an agent for polymerization such as DNA polymerase, reverse transcriptase and the like, and at a suitable temperature and pH. The primer is preferably single stranded for maximum efficiency, but may alternatively be in double stranded form. If  
25       double stranded, the primer is first treated to separate it from its complementary strand before being used to prepare extension products. Preferably, the primer is a polydeoxyribonucleotide.



For review of probe and primer design and annealing conditions, see, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, (2nd ed.), Vols 1-3, Cold Spring Harbor Laboratory, (1989) or Ausebel et al., Current Protocols in Molecular Biology, Greene Publishing and Wiley-Interscience, New York ((1987), the references of which are hereby incorporated. Exemplary hybridization probes and primer pairs are described in Section F1 and in the Examples.

The BDV nucleic acids of this invention, including BDV polynucleotide sequences, as described herein are obtained by conventional nucleic acid procedures, including synthesis as described above, isolation, purification, PCR amplification and the like. Particularly preferred are procedures including those specified for use with the methods of this invention that involve PCR amplification of a provided BDV nucleic acid sample to produce an amplification product containing the nucleotide sequences as described herein.

In preferred embodiments, the nucleic acid sample is enriched for the presence of BDV nucleic acid. Enrichment is typically accomplished by first preparing cDNA from the genomic RNA as described in the Examples followed by PCR amplification employing a PCR primer pair as described herein. The preferred method for obtaining enriched BDV nucleic acid is reverse transcriptase-PCR (RT-PCR) although other

equally useful amplification methods are well known in the art and are applicable with the methods of this invention.

Particularly preferred methods for producing a sample to be assayed use preselected polynucleotides as primers for use in PCR as described in Section F1.

Through the use of recombinant DNA techniques, the BDV nucleic acids described above are used in expression vector systems to produce recombinant BDV polypeptides of this invention. In addition, in an alternative approach, the BDV polypeptides are also obtained by directly synthesized, obtained by purification procedures from particular BDV-polypeptide containing sources, and the like as further described in Section D below.

Exemplary expression vector systems for producing recombinant expressed BDV polypeptides as described below include those that allow expression in prokaryotic and eukaryotic cells. Preferred cells include bacterial, yeast, insect and mammalian cells.

Vectors capable of directing the expression of genes or nucleic acid fragments thereof as well as preselected nucleotide sequences to which they are operably linked are referred to herein as "expression vectors" or "expression plasmids", both of which are also referred to as "plasmids".

As used herein, the term "vector" or "plasmid" refers to a nucleic acid molecule capable of transporting between different genetic environments

another nucleic acid to which it has been operably linked. Thus, plasmids are also referred to as vectors. Preferred vectors are those capable of autonomous replication and expression of structural gene products present in the DNA segments to which they are operably linked. Vectors, therefore, preferably contain the replicons and selectable markers that are compatible with the host selection system.

One type of preferred vector is an episome, i.e., a nucleic acid capable of extra-chromosomal replication. Preferred vectors are those capable of autonomous replication and/or expression of nucleic acids to which they are linked.

A plasmid of this invention is a circular double-stranded plasmid that contains at least a regulation region having elements capable of activating transcription of the translatable BDV polypeptide-encoding nucleotide sequences of this invention. The plasmid further contains a translatable nucleotide sequence from which the desired BDV polypeptides are expressed.

Such expression vectors contain a promotor sequence in the regulatory region which facilitates the efficient transcription of an inserted genetic sequence in the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. The BDV DNA segment can be present in the vector

operably linked to regulatory elements, for example, a promoter (e.g., T7, metallothionein I, or polyhedrin promoters).

In a separate embodiment, a useful, but not  
5 necessary element of an expression vector is one or more selectable or screenable markers. A selectable marker may be a gene that codes for a protein necessary for the survival or growth of a host cell transformed with the vector. The presence of this gene ensures the growth of  
10 only those host cells that contain the vector. Preferred prokaryotic and eukaryotic drug resistance genes respectively confer resistance to ampicillin or tetracyclin and to neomycin (G418 or Geneticin). Typical selection genes encode proteins that (a) confer  
15 resistance to antibiotics or other toxic substances, e.g., chloramphenicol, kanamycin, streptomycin, carbenicillin, mercury, rifampicin, rifampicin, fusaric acid, and the like; (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not  
20 available from complex media, e.g., the gene encoding D-alanine racemase for Bacilli. The choice of the proper selectable marker depends on the host cell, and appropriate markers for different hosts are well known in the art.

25 A screenable marker is a gene that codes for a protein whose activity is easily detected, allowing cells expressing such a marker to be readily identified. Such markers include, for example,  $\beta$ -galactosidase,  $\beta$ -glucuronidase, and luciferase. These markers may be

expressed in the form of a fusion protein with a recombinant BDV polypeptide of this invention as described further below.

5 The choice of vector to which the regulatory region and nucleotide sequences for encoding polypeptides of the present invention is operably linked depends directly, as is well known in the art, on the functional properties desired, e.g., replication or protein expression, and the host cell to be transformed, these being limitations  
10 inherent in the art of constructing recombinant DNA molecules.

The phrase "operably linked" refers to the covalent joining of nucleotide sequences, preferably by conventional phosphodiester bonds, into one strand of  
15 DNA, whether in single or double stranded form. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein- or polypeptide-encoding regions, are contiguous and in proper reading frame.  
20 Moreover, the joining of nucleotide sequences results in the joining of functional elements such as response elements in regulatory regions with promoters and downstream polypeptide-encoding nucleotide sequences as described herein.

25 One typical method for operably linking inserts into expression plasmids is by directional ligation. This is accomplished through a sequence of nucleotides that are adapted for directional ligation. Such a sequence is referred to commonly as a polylinker that is a region of

a DNA expression vector that (1) operably links for replication and transport the upstream and downstream translatable DNA sequences and (2) provides a site or means for directional ligation of a DNA sequence into the vector. Typically, a directional polylinker is a sequence of nucleotides that defines two or more restriction endonuclease recognition sequences, or restriction sites. Upon restriction cleavage, the two sites yield cohesive termini to which a translatable DNA sequence can be ligated to the DNA expression vector. Preferably, the two restriction sites provide, upon restriction cleavage, cohesive termini that are non-complementary and thereby permit directional insertion of a translatable DNA sequence into the cassette.

Techniques for nucleic acid manipulation, such as cloning of nucleic acid sequences encoding BDV polypeptides into expression vectors are described generally, for example, by Sambrook et al., supra, or Ausebel et al., supra, the references of which are hereby incorporated.

A variety of host-expression vector systems may be utilized to express a BDV polypeptide encoded by a nucleotide sequence of this invention. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing a polypeptide-encoding nucleotide sequence; yeast transformed with recombinant yeast expression vectors

containing a polypeptide-encoding nucleotide sequence;  
plant cell systems infected with recombinant virus  
expression vectors (e.g., cauliflower mosaic virus, CaMV;  
tobacco mosaic virus, TMV) or transformed with  
5 recombinant plasmid expression vectors (e.g., Ti plasmid)  
containing a polypeptide-encoding nucleotide sequence;  
insect cell systems infected with recombinant virus  
expression vectors (e.g., baculovirus) containing a  
polypeptide-encoding nucleotide sequence; or animal cell  
10 systems infected with recombinant virus expression  
vectors (e.g., retroviruses, adenovirus, vaccinia virus)  
containing a polypeptide-encoding nucleotide sequence, or  
transformed animal cell systems engineered for stable  
expression. In such cases where glycosylation may be  
15 important, expression systems that provide for  
translational and post-translational modifications may be  
used; e.g., mammalian, insect, yeast or plant expression  
systems.

Depending on the host/vector system utilized, any of  
20 a number of suitable transcription and translation  
elements, including constitutive and inducible promoters,  
transcription enhancer elements, transcription  
terminators, and necessary processing information sites,  
such as ribosome-binding sites, RNA splice sites,  
25 polyadenylation sites, transcriptional terminator  
sequences and the like, may be used in the expression  
vector (see e.g., Bitter, et al., Methods in Enzymology,  
153:516-544, (1987)). Control sequences are chosen so  
that the sequences are functional in the desired host

cell. For the expression in eukaryotic cells, the enhancers or promoters may be those naturally associated with BDV genes, although it will be understood that in many cases others, including those derived from viruses such as SV40, adenovirus, bovine papilloma virus and the like, are equally appropriate.

For example, when cloning in bacterial systems, inducible promoters such as P<sub>l</sub> of bacteriophage  $\lambda$ , P<sub>lac</sub>, P<sub>trp</sub>, P<sub>tac</sub> (P<sub>trp</sub>-lac hybrid promoter) and the like may be used. When cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) may be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of a polypeptide-encoding nucleotide sequence.

In bacterial systems a number of expression vectors may be advantageously selected for expression of a polypeptide-encoding nucleotide sequence according to the methods of this invention. For example, when large quantities are to be produced, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Those which are engineered to contain a cleavage site to aid in recovering the protein are preferred. Such vectors include but are not limited to the E. coli expression vector pUR278 (Ruther, et al., EMBO J., 2:1791, (1983)),



in which a polypeptide-encoding nucleotide sequence of this invention may be ligated into the vector in frame with the lacZ coding region so that a hybrid polypeptide-lacZ protein is produced; pIN vectors (Inouye & Inouye, Nuc. Acids Res., 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem., 264:5503-5509 (1989)); and the like.

In one embodiment, the vector utilized includes prokaryotic sequences that facilitate the propagation of the vector in bacteria, i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extra-chromosomally when introduced into a bacterial host cell. Such replicons are well known in the art.

Those vectors that include a prokaryotic replicon also typically include convenient restriction sites for insertion of a recombinant DNA molecule of the present invention. Typical of such vector plasmids are pUC18, pUC19, pBR322, and pBR329 available from BioRad Laboratories, (Richmond, CA) and pPL available from Pharmacia, (Piscataway, NJ), and pBLUESCRIPT and pBS available from Stratagene, (La Jolla, CA). A vector of the present invention may also be a Lambda phage vector including those Lambda vectors described in Molecular Cloning: A Laboratory Manual, Second Edition, Maniatis et al., eds., Cold Spring Harbor, NY (1989).

In another preferred embodiment, plasmid vectors for use in the present invention are also compatible with eukaryotic cells. Eukaryotic cell expression vectors are

well known in the art and are available from several commercial sources. Typically, such vectors provide convenient restriction sites for insertion of the desired recombinant DNA molecule, and further contain promoters  
5 for expression of the encoded genes which are capable of expression in the eukaryotic cell, as discussed earlier. Typical of such vectors are pSVO and pKSV-10 (Pharmacia), and pPVV-1/PML2d (International Biotechnology, Inc.), and pTDT1 (ATCC, No. 31255).

10 In addition, in eukaryotic plasmids, one or more transcription units are present that are expressed only in eukaryotic cells. The eukaryotic transcription unit consists of noncoding sequences and sequences encoding selectable markers. The expression vectors of this  
15 invention also contain distinct sequence elements that are required for accurate and efficient polyadenylation. In addition, splicing signals for generating mature mRNA are included in the vector. The eukaryotic plasmid expression vectors contain viral replicons, the presence  
20 of which provides for the increase in the level of expression of cloned genes. A preferred replication sequence is provided by the simian virus 40 or SV40 papovavirus.

25 A variety of yeast cultures and suitable expression vectors for transforming yeast cells are known for use in this invention. See, e.g., U.S. Patents 4,745,057, 4,797,359, 4,615,974, 4,880,734, 4,711,844, and 4,865,989, the disclosures of which are hereby incorporated by reference.

An alternative expression system that can be used to express a protein of the invention is an insect system. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. A BDV polypeptide-encoding nucleotide sequence may be cloned into non-essential regions (in *Spodoptera frugiperda* for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the polypeptide-encoding nucleotide sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect cells in which the inserted gene is expressed. See Smith, et al., J. Biol. Chem., 46:584 (1983); Smith, U.S. Patent No. 4,215,051.

Mammalian cell systems that utilize recombinant viruses or viral elements to direct expression may be engineered. For example, when using adenovirus expression vectors, the coding sequence of a BDV polypeptide may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted into the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable

and capable of expressing the polypeptide in infected hosts (e.g., see Logan et al., Proc. Natl. Acad. Sci., USA, 81:3655-3659 (1984)). Alternatively, the vaccinia virus 7.5K promoter may be used, e.g., see, Mackett, et al., Proc. Natl. Acad. Sci., USA, 79:7415-7419 (1982); Mackett, et al., J. Virol., 49:857-864 (1984); Panicali, et al., Proc. Natl. Acad. Sci., USA, 79:4927-4931 (1982)). Of particular interest are vectors based on bovine papilloma virus which have the ability to replicate as extrachromosomal elements (Sarver, et al., Mol. Cell. Biol., 1:486 (1981)). Shortly after entry of this DNA into mouse cells, the plasmid replicates to about 100 to 200 copies per cell. Transcription of the inserted cDNA does not require integration of the plasmid into the host's chromosome, thereby yielding a high level of expression. These vectors can be used for stable expression by including a selectable marker in the plasmid, such as the neo gene. Alternatively, the retroviral genome can be modified for use as a vector capable of introducing and directing the expression of a BDV polypeptide-encoding nucleotide sequence in host cells (Cone et al., Proc. Natl. Acad. Sci., USA, 81:6349-6353 (1984)). High level expression may also be achieved using inducible promoters, including, but not limited to, the metallothionine IIA promoter and heat shock promoters.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. Expression vectors containing viral origins of replication or other

appropriate expression control elements (e.g., promoter and enhancer sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker are useful for stably transforming host cells. As mentioned above, the selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines.

Both prokaryotic and eukaryotic expression vectors are familiar to one of ordinary skill in the art of vector construction and are described by Ausebel, et al., In Current Protocols in Molecular Biology, Wiley and Sons, New York (1993) and by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, (1989).

Expression vectors may also include secretion signals, which allow the protein to cross the cell membrane and either pass completely out of the cell permitting more convenient purification, or else lodge in cell membranes, and thus attain its functional topology.

The vectors of this invention may also be selected for producing BDV polypeptides in the form of a fusion polypeptide. Such vectors and use thereof are well known in the art and are described in Ausebel, et al., In Current Protocols in Molecular Biology, Wiley and Sons, New York (1993) and by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, (1989).

Depending upon the selected expression vector, compatible host cells are then selected. For prokaryotic expression, host cells include bacterial cells such as E. coli or B. subtilis. For eukaryotes, host cells may include filamentous fungi, plant cells, insect cells and mammalian cells. The latter are often used for the expression of polypeptides derived from eukaryotes.

The term "transformed" refers to the state of a host cell having received a vector containing the nucleic acids of interest. Transformation methods, which vary depending on the type of host cell, include the following: electroporation; transfection employing calcium chloride, DEAE-dextran and the like; lipofection; infection where the vector is an infectious agent; and other methods. The transformed cells also include the progeny of such cells.

#### D. Human BDV Polypeptides

One aspect of the present invention includes substantially purified BDV polypeptides that are encoded by the human BDV nucleic acids described in Section C. Accordingly, the phrase "BDV polypeptide" refers to a polypeptide having an amino acid residue sequence that comprises an amino acid residue sequence that corresponds, and preferably is identical, to a portion of a BDV molecule isolated from a human. In particular, a BDV polypeptide comprises an amino acid residue sequence that is encoded by and deduced from the BDV nucleic acids

isolated from the three patients, H1, H2 and H3, as described in Section B.

Thus, a BDV polypeptide includes an amino acid residue sequence of an entire human BDV genome, or a portion thereof. A BDV polypeptide includes those that are either derived from a naturally occurring BDV polypeptide or are synthetic or recombinant as described herein. The latter non-natural BDV polypeptides share significant structural and functional characteristics peculiar to a naturally occurring BDV polypeptide of the present invention. Within the definition of a BDV polypeptide and corresponding encoding nucleic acid, are BDV isotypes, strains and related viruses. A BDV protein or polypeptide includes the full-length encoded protein of a particular BDV ORF, fragments thereof, proteins containing immunoepitopes of the BDV polypeptides of this invention, and functional equivalents of the foregoing. The phrase "substantially purified" and its equivalents with regard to BDV polypeptides is described in Section A. Essentially, a polypeptide is substantially purified when it is separated from the native contaminants which accompany it in its natural state. Thus, a polypeptide which is purified from a body sample, chemically synthesized or synthesized by recombinant DNA techniques will be substantially free from the naturally-associated components found in each of those environments.

Preferred polypeptide regions in the BDV genome are those complete polypeptides or portions thereof that are encoded by the open reading frames (ORF) present in the

BDV genome. ORF encode the p24, p16, p56, p40 and L polymerase polypeptides as more fully described in the Examples. Particularly preferred p24 polypeptides encoded by full-length ORF p24 are shown in Figures 8A, 8B and 8C that are also respectively listed as SEQ ID NO 20, SEQ ID NO 21 and SEQ ID NO 22. Also preferred are p24 polypeptide fragments containing at least the amino acid sequence, presented in single letter code, MATGPSSLVDSLEDEEDP (SEQ ID NO 32) and RIYPQLPSAPTADEWDIIP (SEQ ID NO 33).

Similarly, preferred p16 polypeptides encoded by full-length p16 ORF are shown in Figures 9A, 9B and 9C that are also respectively listed as SEQ ID NO 23, SEQ ID NO 24 and SEQ ID NO 25. Also preferred are p16 polypeptide fragments containing at least the amino acid sequence, presented in single letter code, MNSKHSYVELKGKVIVPG (SEQ ID NO 34) and RLRNIGVGPLGPDIRSSGP (SEQ ID NO 35).

Also preferred are the p56 polypeptides encoded by full-length p56 ORF shown in Figures 10A and 10B that are also respectively listed as SEQ ID NO 26 and SEQ ID NO 27. Further preferred are p56 polypeptide fragments containing at least the amino acid sequence, presented in single letter code, GLSCNTDSTPGLIDLEIR (SEQ ID NO 36), RSKLRRRRRDTQQIEYLV (SEQ ID NO 37) and LISLCVSLPASFARRRRLGRWQE (SEQ ID NO 38).

A further preferred human BDV p40 full-length polypeptide encoded by p40 ORF has an amino acid residue sequence shown in Figures 11A, 11B and 11C that are also



respectively listed as SEQ ID NO 28, SEQ ID NO 29, SEQ ID NO 30. Further preferred are p40 polypeptide fragments containing at least the amino acid sequence, presented in single letter code MPPKRRLVDDADAMEDQD (SEQ ID NO 39),  
5 MEDQDDLYEPPSSLPKLP (SEQ ID NO 40) and ELSGEISAIMRMIGLTGLN (SEQ ID NO 41).

Another preferred human BDV polypeptide is the catalytic domain polypeptide from L polymerase having an amino acid residue sequence shown in Figure 12 and as  
10 listed in SEQ ID NO 31.

In one aspect, a BDV polypeptide of this invention is characterized by having the capacity to immunoreact with BDV antibodies raised in response to BDV infection in a subject for use in detecting BDV antigens in a  
15 sample. In one embodiment, such detection is useful for diagnosing subjects suspected of having BDV for either confirming or negating BDV as the infectious agent as further described in the Methods Sections below. Preferably, a BDV polypeptide of this invention is  
20 further characterized by its ability to immunologically mimic an epitope (antigenic determinant) expressed by BDV. Such a BDV epitope will allow immunologic detection of the virus or polypeptide in a physiological sample with reasonable assurance. Usually, although not in all  
25 cases, it will be desirable that the epitope be immunologically distinct from viruses other than BDV to allow for differential diagnosis of viral presence. Such a polypeptide is useful herein as a component in an inoculum for producing antibodies that immunoreact with

native BDV protein for use in detecting the presence of such protein for use in the methods of this invention.

As used herein, the phrase "immunologically mimic" in its various grammatical forms refers to the ability of a BDV polypeptide of this invention to immunoreact with an antibody of the present invention that recognizes a conserved native epitope of BDV as defined herein.

It should be understood that a subject polypeptide need not be identical to the amino acid residue sequence of the three human BDV amino acid sequences encoded by the respective patient-specific isolated nucleic acids, so long as the polypeptides are able to bind to patient-derived anti-BDV antibodies and are able to generate anti-polypeptide antibodies that are useful in detecting BDV polypeptides in a subject, both of which are as described in Section F.

A subject polypeptide includes any analog, fragment or chemical derivative of a polypeptide whose amino acid residue sequence is shown herein so long as the polypeptide has the characteristics described herein. Therefore, a present polypeptide can be subject to various changes, substitutions, insertions, and deletions where such changes provide for certain advantages in its use. In this regard, a BDV polypeptide of this invention corresponds to, rather than is identical to, the sequence of BDV where one or more changes are made and it retains the ability to immunoreact with anti-BDV antibodies and can be used as an immunogen to generate antibodies that immunoreact with BDV polypeptides.

The term "analog" includes any polypeptide having an amino acid residue sequence substantially identical to a sequence specifically shown herein in which one or more residues have been conservatively substituted with a functionally similar residue and which displays the aforementioned characteristics. Examples of conservative substitutions include the substitution of one non-polar (hydrophobic) residue such as isoleucine, valine, leucine or methionine for another, the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, between glycine and serine, the substitution of one basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another.

The phrase "conservative substitution" also includes the use of a chemically derivatized residue in place of a non-derivatized residue provided that such polypeptide displays the requisite activities.

"Chemical derivative" refers to a subject polypeptide having one or more residues chemically derivatized by reaction of a functional side group. Such derivatized molecules include for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides.

Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine may be derivatized to form N-im-benzylhistidine. Also included as chemical derivatives are those peptides which contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids. For examples: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine. Polypeptides of the present invention also include any polypeptide having one or more additions and/or deletions or residues relative to the sequence of a polypeptide whose sequence is shown herein, so long as the requisite activity is maintained.

The term "fragment" refers to any subject polypeptide having an amino acid residue sequence shorter than that of a polypeptide whose amino acid residue sequence is shown herein.

When a BDV polypeptide has a sequence that is not identical to the sequence of human BDV of this invention, it is typically because one or more conservative or non-conservative substitutions have been made, usually no more than about 30 number percent, more usually no more than 20 number percent, and preferably no more than 10 number percent of the amino acid residues are substituted. Additional residues may also be added at either terminus for the purpose of providing a "linker" by which the polypeptides of this invention can be

conveniently affixed to a label or solid matrix, or carrier. Preferably the linker residues do not form BDV epitopes, i.e., are not similar in structure to BDV.

5 Labels, solid matrices and carriers that can be used with the polypeptides of this invention are described hereinbelow.

10 Amino acid residue linkers are usually at least one residue and can be 40 or more residues, more often 1 to 10 residues, but do not form BDV epitopes. Typical amino acid residues used for linking are tyrosine, cysteine, lysine, glutamic and aspartic acid, or the like. In addition, a subject polypeptide can differ, unless otherwise specified, from the natural sequence of BDV by the sequence being modified by terminal-NH<sub>2</sub> acylation, 15 e.g., acetylation, or thioglycolic acid amidation, by terminal-carboxlyamidation, e.g., with ammonia, methylamine, and the like.

20 When coupled to a carrier to form what is known in the art as a carrier-hapten conjugate, a BDV polypeptide of the present invention is capable of inducing antibodies that immunoreact with BDV in a subject suspected of having BDV. In view of the well established principle of immunologic crossreactivity, the present invention therefore contemplates antigenically related 25 variants of the polypeptides described herein. An "antigenically related variant" is a subject polypeptide that is capable of inducing antibody molecules that immunoreact with a BDV polypeptide fragment and with BDV.

Any peptide of the present invention may be used in the form of a pharmaceutically acceptable salt. Suitable acids which are capable of forming salts with the peptides of the present invention include inorganic acids  
5 such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, phosphoric acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, anthranilic  
10 acid, cinnamic acid, naphthalene sulfonic acid, sulfanilic acid or the like.

Suitable bases capable of forming salts with the peptides of the present invention include inorganic bases such as sodium hydroxide, ammonium hydroxide, potassium  
15 hydroxide and the like; and organic bases such as mono-, di- and tri-alkyl and aryl amines (e.g., triethylamine, diisopropyl amine, methyl amine, dimethyl amine and the like) and optionally substituted ethanolamines (e.g., ethanolamine, diethanolamine and the like).

20 A BDV polypeptide of the present invention, also referred to herein as a subject polypeptide, can be synthesized by any of the techniques that are known to those skilled in the polypeptide art, including recombinant DNA techniques. Synthetic chemistry  
25 techniques, such as a solid-phase Merrifield-type synthesis, are preferred for reasons of purity, antigenic specificity, freedom from undesired side products, ease of production and the like. An excellent summary of the many techniques available can be found in J.M. Steward

and J.D. Young, "Solid Phase Peptide Synthesis", W.H. Freeman Co., San Francisco, 1969; M. Bodanszky, et al., "Peptide Synthesis", John Wiley & Sons, Second Edition, 1976 and J. Meienhofer, "Hormonal Proteins and Peptides", Vol. 2, p. 46, Academic Press (New York), 1983 for solid phase peptide synthesis, and E. Schroder and K. Kubke, "The Peptides", Vol. 1, Academic Press (New York), 1965 for classical solution synthesis, each of which is incorporated herein by reference. Appropriate protective groups usable in such synthesis are described in the above texts and in J.F.W. McOmie, "Protective Groups in Organic Chemistry", Plenum Press, New York, 1973, the disclosure of which is incorporated herein by reference.

In general, the solid-phase synthesis methods contemplated comprise the sequential addition of one or more amino acid residues or suitably protected amino acid residues to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid residue is protected by a suitable, selectively removable protecting group. A different, selectively removable protecting group is utilized for amino acids containing a reactive side group such as lysine.

Using a solid phase synthesis as exemplary, the protected or derivatized amino acid is attached to an inert solid support through its unprotected carboxyl or amino group. The protecting group of the amino or carboxyl group is then selectively removed and the next amino acid in the sequence having the complimentary (amino or carboxyl) group suitably protected is admixed

and reacted under conditions suitable for forming the amide linkage with the residue already attached to the solid support. The protecting group of the amino or carboxyl group is then removed from this newly added amino acid residue, and the next amino acid (suitably protected) is then added, and so forth. After all the desired amino acids have been linked in the proper sequence, any remaining terminal and side group protecting groups (and solid support) are removed sequentially or concurrently, to afford the final polypeptide.

Alternatively, a BDV polypeptide may be purified from a sample such as a cell or fluid. Both purification of a BDV polypeptide in a cell lysate as well as body fluid containing secreted viral proteins are contemplated. The natural BDV polypeptides may be isolated from the whole virus by conventional techniques, such as affinity chromatography. Conveniently, polyclonal or monoclonal antibodies obtained according to the present invention may be used to prepare a suitable affinity column by well known techniques. See, for example, Hudson and May, Practical Immunology, Blackwell Scientific Publications, Oxford, United Kingdom (1980), the disclosure of which is hereby incorporated by reference. A BDV polypeptide may also be purified to substantial homogeneity by standard techniques well known in the art, including selective precipitation, column chromatography, and the like.



With purification methods, a BDV polypeptide of the present invention will be typically from about 50% or more pure, preferably at least 80% pure, and more preferably, at least 95% pure. Using conventional techniques of protein purification, homogenous polypeptide compositions of at least about 99% can be obtained.

A preferred method for producing a BDV polypeptide, other than direct purification from a body sample such as a cell lysate or fluid sample, involves the expression in host cells of recombinant DNA molecules in which a BDV nucleic acid encodes a desired portion, whether synthetic or natural, of the BDV genome as discussed in Section C. Preferred expression vectors and BDV nucleic acids are described above for expressing BDV recombinant polypeptides as previously discussed. The expressed BDV polypeptide is thus referred to as a BDV recombinant polypeptide. If the expression vector selected for use is designed to operatively link the BDV polypeptide to another molecule, such as GST or MBP, a fusion polypeptide is expressed that contains BDV linked thereto. The latter compositions are useful in purification of expressed fusion polypeptides. Thereafter, the expressed fusion polypeptide is used in accordance with the methods of the invention or can be separated from the other molecule in the fusion polypeptide.

A BDV polypeptide, purified, synthesized or expressed, can be used, inter alia, in the diagnostic

methods and systems of the present invention to detect BDV and antibodies thereto present in a sample, or can be used to prepare an inoculum as described herein for the preparation of antibodies that immunoreact with conserved epitopes on BDV.

E. Human BDV Anti-Polypeptide Antibodies

The term "antibody" in its various grammatical forms is used herein as a collective noun that refers to a population of immunoglobulin molecules and/or immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antibody combining site or paratope.

An "antibody combining site" is that structural portion of an antibody molecule comprised of heavy and light chain variable and hypervariable regions that specifically binds antigen.

The phrase "antibody molecule" in its various grammatical forms as used herein contemplates both an intact immunoglobulin molecule and an immunologically active portion of an immunoglobulin molecule.

Exemplary antibody molecules for use in the diagnostic methods and systems of the present invention are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contain the paratope, including those portions known in the art as Fab, Fab',  $F(ab')_2$  and F(v).

Fab and  $F(ab')_2$  portions of antibodies are prepared by the proteolytic reaction of papain and pepsin, respectively, on substantially intact antibodies by methods that are well known. See for example, U.S.

5 Patent No. 4,342,566 to Theofilopolous and Dixon. Fab' antibody portions are also well known and are produced from  $F(ab')_2$  portions followed by reduction of the disulfide bonds linking the two heavy chain portions as with mercaptoethanol, and followed by alkylation of the  
10 resulting protein mercaptan with a reagent such as iodoacetamide. An antibody containing intact antibody molecules are preferred, and are utilized as illustrative herein.

The phrase "substantially purified" means that the  
15 antibody molecules do not immunoreact with the stated antigen at levels within one order of magnitude, and preferably within two orders of magnitude, of the level of immunoreaction with a species of antigen recited to immunoreact with the antibody molecule when  
20 immunoreaction is expressed as an equilibrium constant between bound (immunoreacted) and nonbound antigen.

An antibody of the present invention, i.e., an anti-BDV antibody, in one embodiment is characterized as comprising antibody molecules that immunoreact with human  
25 BDV proteins and polypeptides thereof as described in Section D. In other embodiments, an anti-BDV antibody is characterized as being capable of immunoreacting with any species of BDV protein or polypeptide or fragments thereof.

Antibody immunoreactivity with BDV-containing antigens can be measured by a variety of immunological assays known in the art. Exemplary immunoreaction of an anti-BDV antibody with a BDV polypeptide is described in  
5 Section F and in the Examples.

An antibody of the present invention is typically produced by immunizing a mammal with an inoculum containing an BDV polypeptide of this invention and thereby induce in the mammal antibody molecules having  
10 immunospecificity for immunized BDV polypeptide. The antibody molecules are then collected from the mammal and isolated to the extent desired by well known techniques such as, for example, by using DEAE Sephadex to obtain the IgG fraction.

15 The preparation of antibodies against a polypeptide is well known in the art. [See Staudt et al., J. Exp. Med., 157:687-704 (1983)]. Briefly, to produce a peptide antibody composition of this invention, a laboratory mammal is inoculated with an immunologically effective  
20 amount of a BDV polypeptide, typically as described in the Examples. The anti-BDV polypeptide antibody molecules thereby induced are then collected from the mammal and those immunospecific for both the BDV polypeptide and isolated BDV are isolated to the extent  
25 desired by well known techniques such as, for example, by immunoaffinity chromatography.

To enhance the specificity of the antibody, the antibodies are preferably purified by immunoaffinity chromatography using solid phase-affixed immunizing

polypeptide. The antibody is contacted with the solid phase-affixed immunizing polypeptide for a period of time sufficient for the polypeptide to immunoreact with the antibody molecules to form a solid phase-affixed immunocomplex. The bound antibodies are separated from the complex by standard techniques.

The word "inoculum" or "immunogen" in its various grammatical forms is used herein to describe a composition containing a BDV polypeptide of this invention as an active ingredient used for the preparation of antibodies against an BDV polypeptide. When a polypeptide is used in an inoculum to induce antibodies it is to be understood that the polypeptide can be used in various embodiments, e.g., alone or linked to a carrier as a conjugate, or as a polypeptide polymer. However, for ease of expression and in context of a polypeptide inoculum, the various embodiments of the polypeptides of this invention are collectively referred to herein by the term "polypeptide" and its various grammatical forms.

For a polypeptide that contains fewer than about 35 amino acid residues, it is preferable to use the peptide bound to a carrier for the purpose of inducing the production of antibodies.

One or more additional amino acid residues can be added to the amino- or carboxy-termini of the polypeptide to assist in binding the polypeptide to a carrier. Cysteine residues added at the amino- or carboxy-termini of the polypeptide have been found to be particularly

useful for forming conjugates via disulfide bonds.

However, other methods well known in the art for preparing conjugates can also be used.

The techniques of polypeptide conjugation or coupling through activated functional groups presently known in the art are particularly applicable. See, for example, Aurameas, et al., Scand. J. Immunol., Vol. 8, Suppl. 7:7-23 (1978) and U.S. Patent No. 4,493,795, No. 3,791,932 and No. 3,839,153. In addition, a site-directed coupling reaction can be carried out so that any loss of activity due to polypeptide orientation after coupling can be minimized. See, for example, Rodwell et al., Biotech., 3:889-894 (1985), and U.S. Patent No. 4,671,958.

Exemplary additional linking procedures include the use of Michael addition reaction products, di-aldehydes such as glutaraldehyde, Klipstein, et al., J. Infect. Dis., 147:318-326 (1983) and the like, or the use of carbodiimide technology as in the use of a water-soluble carbodiimide to form amide links to the carrier. Alternatively, the heterobifunctional cross-linker SPDP (N-succinimidyl-3-(2-pyridyldithio) proprionate)) can be used to conjugate peptides, in which a carboxy-terminal cysteine has been introduced.

Useful carriers are well known in the art, and are generally proteins themselves. Exemplary of such carriers are keyhole limpet hemocyanin (KLH), edestin, thyroglobulin, albumins such as bovine serum albumin (BSA) or human serum albumin (HSA), red blood cells such

as sheep erythrocytes (SRBC), tetanus toxoid, cholera toxoid as well as polyamino acids such as poly D-lysine:D-glutamic acid, and the like.

5 The choice of carrier is more dependent upon the ultimate use of the inoculum and is based upon criteria not particularly involved in the present invention. For example, a carrier that does not generate an untoward reaction in the particular animal to be inoculated should be selected.

10 The present inoculum contains an effective, immunogenic amount of a polypeptide of this invention, typically as a conjugate linked to a carrier. The effective amount of polypeptide per unit dose sufficient to induce an immune response to the immunizing  
15 polypeptide depends, among other things, on the species of animal inoculated, the body weight of the animal and the chosen inoculation regimen is well known in the art. Inocula typically contain polypeptide concentrations of about 10 micrograms ( $\mu\text{g}$ ) to about 500 milligrams (mg) per  
20 inoculation (dose), preferably about 50 micrograms to about 50 milligrams per dose.

25 The term "unit dose" as it pertains to the inocula refers to physically discrete units suitable as unitary dosages for animals, each unit containing a predetermined quantity of active material calculated to produce the desired immunogenic effect in association with the required diluent, i.e., carrier, or vehicle. The specifications for the novel unit dose of an inoculum of this invention are dictated by and are directly dependent

on (a) the unique characteristics of the active material and the particular immunologic effect to be achieved, and (b) the limitations inherent in the art of compounding such active material for immunologic use in animals, as disclosed in detail herein, these being features of the present invention.

Inocula are typically prepared from the dried solid polypeptide-conjugate by dispersing the polypeptide-conjugate in a physiologically tolerable (acceptable) diluent such as water, saline or phosphate-buffered saline to form an aqueous composition.

Inocula can also include an adjuvant as part of the diluent. Adjuvants such as complete Freund's adjuvant (CFA), incomplete Freund's adjuvant (IFA) and alum are materials well known in the art, and are available commercially from several sources.

The antibody so produced can be used, inter alia, in the diagnostic methods and systems of the present invention as described in Section F and in the Examples.

A preferred anti-BDV antibody is a monoclonal antibody and is used herein as exemplary of an anti-BDV antibody.

The phrase "monoclonal antibody" in its various grammatical forms refers to a population of antibody molecules that contain only one species of antibody combining site capable of immunoreacting with a particular epitope. A monoclonal antibody thus typically displays a single binding affinity for any epitope with which it immunoreacts. A monoclonal antibody may contain



an antibody molecule having a plurality of antibody combining sites, each immunospecific for a different epitope, e.g., a bispecific monoclonal antibody.

5 A monoclonal antibody of this invention comprises antibody molecules that bind to human BDV protein and polypeptides derived thereof.

10 A monoclonal antibody is typically composed of antibodies produced by clones of a single cell called a hybridoma that secretes (produces) only one kind of antibody molecule. The hybridoma cell is formed by fusing an antibody-producing cell and a myeloma or other self-perpetuating cell line. The preparation of such antibodies was first described by Kohler and Milstein, Nature, 256:495-497 (1975), the description of which is  
15 incorporated by reference. The hybridoma supernates so prepared can be screened for the presence of antibody molecules that immunoreact with a BDV polypeptide.

20 Briefly, to form the hybridoma from which the monoclonal antibody composition is produced, a myeloma or other self-perpetuating cell line is fused with lymphocytes obtained from the spleen of a mammal hyperimmunized with a BDV antigen, such as is present in a PS polypeptide of this invention. The polypeptide-induced hybridoma technology is described by Niman et al., Proc. Natl. Acad. Sci., USA, 80:4949-4953 (1983),  
25 the description of which is incorporated herein by reference.

It is preferred that the myeloma cell line used to prepare a hybridoma be from the same species as the

lymphocytes. Typically, a mouse of the strain 129 GlX<sup>+</sup> is the preferred mammal. Suitable mouse myelomas for use in the present invention include the hypoxanthine-aminopterin-thymidine-sensitive (HAT) cell lines P3X63-Ag8.653, and Sp2/0-Ag14 that are available from the American Type Culture Collection, Rockville, MD, under the designations CRL 1580 and CRL 1581, respectively.

Splenocytes are typically fused with myeloma cells using polyethylene glycol (PEG) 1500. Fused hybrids are selected by their sensitivity to HAT. Hybridomas producing a monoclonal antibody of this invention are identified using the enzyme linked immunosorbent assay (ELISA), well known to those of ordinary skill in the art.

A monoclonal antibody of the present invention can also be produced by initiating a monoclonal hybridoma culture comprising a nutrient medium containing a hybridoma that produces and secretes antibody molecules of the appropriate polypeptide specificity. The culture is maintained under conditions and for a time period sufficient for the hybridoma to secrete the antibody molecules into the medium. The antibody-containing medium is then collected. The antibody molecules can then be further isolated by well known techniques.

Media useful for the preparation of these compositions are both well known in the art and commercially available and include synthetic culture media, inbred mice and the like. An exemplary synthetic medium is Dulbecco's Minimal Essential Medium (DMEM;

Dulbecco et al., Virology 8:396 (1959)) supplemented with 4.5 gm/1 glucose, 20 mm glutamine, and 20% fetal calf serum. An exemplary inbred mouse strain is the Balb/c.

Other methods of producing a monoclonal antibody, a hybridoma cell, or a hybridoma cell culture are also well known. See, for example, the method of isolating monoclonal antibodies from an immunological repertoire as described by Sastry, et al., Proc. Natl. Acad. Sci., USA, 86:5728-5732 (1989); and Huse et al., Science, 246:1275-1281 (1989). Thus, recombinant BDV antibodies are also contemplated for use in this invention. Recombinant human BDV antibodies include those against BDV polypeptides as well as those generated in a human in response to BDV infection. For the latter, recombinant antibodies are prepared once a nucleic acid encoding such an antibody has been isolated from a human and cloned. Thus, the nucleic acid segments that encode these antibodies are a further part of the invention. These segments can be used in expression vectors as described in Section C for the high level expression of a BDV antibody sequence and as desired, the collection and purification of the light chains, heavy chains, light/heavy chain dimers or intact antibodies, binding fragments, or any other forms such as single chain constructs that may follow.

The monoclonal antibodies of this invention can be used in the same manner as disclosed herein for antibodies of the present invention.

Also contemplated by this invention is the hybridoma cell, and cultures containing a hybridoma cell that produce a monoclonal antibody of this invention.

5        F.    Diagnostic Methods

         1.    Detecting Human BDV Nucleic Acids

         In one aspect of the present invention, the presence of BDV nucleic acid is detected in a sample. While preferred, the detection of a BDV nucleic acid is not limited to humans, as the nucleic acid molecules and methods of this invention allow for the detection of related BDV species, isotypes and strains, and variants thereof. Thus, the methods of this invention are useful for detecting ongoing or past BDV infection via the detection of BDV nucleic acids, diseases caused by BDV, related viruses or variants thereof, as well as to eliminate the suspicion of BDV viral infection, either past or ongoing. The detecting of BDV is generally accomplished by hybridizing the nucleic acid in the sample with a BDV nucleic acid molecule of this invention or a region derived therefrom.

         In one embodiment, the sample is a biological sample, tissues, cells, lysates thereof or body fluids, obtained from a subject suspected of having BDV. As such, the body sample is either isolated BDV-infectable cells or fluid samples containing BDV viral particles. A preferred isolatable BDV-infected cell type is peripheral blood mononuclear cells (PBMC). As described in the Examples, BDV-infectable cells are also contemplated to

be cell lines that become infected with BDV following co-cultivation with BDV-infected subject-derived cells. A preferred BDV-infectable cell line is a human oligodendroglial cell line. Other BDV-infectable cell lines are also contemplated for use in the methods of this invention. In alternative embodiments, the sample is a laboratory sample including but not limited to cells, transfected or infected, lysates thereof or culture fluids, that may contain a BDV nucleic acid.

In detecting BDV nucleic acid, a nucleic acid molecule is designed for use in the hybridization methods as described herein to hybridize to a target BDV sequence in a sample. As defined herein, a "nucleic acid molecule" is a nucleotide sequence that is derived from a particular region of BDV genome. The specific sequence is dependent upon the desired BDV target sequence in the sample.

Since hybridization methods as described herein rely upon the substantial complementarity of a nucleic acid molecule to the target BDV sequence, the sequence of a particular nucleic acid molecule is so determined. The BDV sequence of the nucleic acid molecule may be derived from any known sequence of BDV, such as the C6BV strain or Strain V that were originally obtained in horses then passaged through rodents. Preferably, the nucleic acid molecules have a nucleotide sequence corresponding to a BDV sequence determined from human BDV isolates. Thus, as defined herein, the phrase "derived from" means that the nucleic acid molecule is substantially complementary

to one strand of a known BDV nucleic acid or substantially identical to the other strand such that specific hybridization with the target human BDV nucleic acid in the body sample is obtained. Therefore, "derived  
5 from" is used to indicate a relative correspondence with the desired target sequence.

The sequence of such nucleic acid molecules need not have perfect complementarity with the BDV genome or cDNA synthesized therefrom, as long as substantial

10 complementarity is maintained. The nucleic acid molecule may be derived from any region or portion of the selected target sequence with the only requirement being that the molecule be of sufficient length to allow for specific hybridization as defined in Section B1. Preferably, the  
15 molecule is at least 5 nucleotides in length, more preferably 10 nucleotides in length and most preferably, between 15 and 25 nucleotides in length. However, in certain situations, longer molecules are desirable, such as the full-length BDV human cDNAs of this invention.

20 The specific length that is selected is dependent on the type of hybridization method used to screen a sample. As known to one skilled in the art, the conditions for hybridization, based on stringency of hybridization principles, varies with the length of the nucleic acid  
25 molecule.

Preferred human BDV genomes from which nucleic acid molecules are derived are those identified in the three BDV patients of this invention, as described herein and in the Examples. As more patient- or subject-specific

sequences become available, nucleic acid molecules for use in the methods of this invention are similarly derived.

5 Detecting the presence of BDV nucleic acid in any sample, including a patient sample, may be performed by hybridization with the nucleotide region encoding a BDV polypeptide of this invention. For example, a BDV nucleotide encoding the p24 polypeptide corresponding to patients H1 (SEQ ID NO 20), H2 (SEQ ID NO 21) and H3 (SEQ  
10 ID NO 22) and polypeptides derived therefrom as described in Section D is one of the preferred embodiments. A preferred screening nucleic acid molecule sequence used is the p24 nucleotide sequence shown in SEQ ID NO 3, SEQ ID NO 4 and SEQ ID NO 5, or regions derived therefrom,  
15 that respectively correspond to the p24 sequence from patients H1, H2 and H3. For example, in particular, a preferred p24 nucleic acid for use in the methods described herein is a substantially purified nucleic acid encoding a human Born disease virus (BDV) p24  
20 polypeptide comprising an amino acid residue sequence selected from the group consisting of SEQ ID NO 20, SEQ ID NO 21, SEQ ID NO 22, MATGPSSLVDSLEDEEDP (SEQ ID NO 32) and RIYPQLPSAPTADEWDIIP (SEQ ID NO 33). Other BDV nucleic acids in a sample corresponding to p16, p56, p40  
25 and the catalytic domain are similarly identified with the human nucleic acids encoding the respective polypeptides as described in Sections D and E, respectively, and incorporated herein for use in the

methods of this invention. As described below, the nucleic acid molecules are used as primers and probes.

In one aspect of detecting BDV nucleic acids, hybridizing is performed with the polymerase chain reaction (PCR). Depending on the region of BDV to be amplified, particular primers are selected as described below. Preferably the sample of nucleic acid from the subject is first converted to cDNA with methods well known in the art. Exemplary methods of preparing cDNA from BDV genomic RNA are described in the Examples.

The genetic material to be assayed is first denatured, typically by melting, to eliminate structures that may interfere with the synthesis of cDNA. The nucleic acid, preferably a cDNA, is subjected to a PCR amplification by treating (contacting) the sample with a PCR primer pair, each member of the pair having a preselected nucleotide sequence based on the design requirements as described in Section C above. Primers comprising a primer pair, having first and second primers, are capable of initiating a primer extension reaction by hybridizing to a template nucleotide sequence, preferably at least about 10 nucleotides in length, more preferably between 15 nucleotides in length and 25 nucleotides in length, that are present and preferably conserved within a BDV nucleotide segment template.

The first primer of a PCR pair is sometimes referred to as the "sense" primer because it is derived from the sense (coding strand or positive sense strand) and it



hybridizes to the anti-sense (non-coding or negative sense strand) of a nucleic acid, i.e., a strand complementary to a coding strand. The first primer is also referred to as a forward or 5' primer. Accordingly, the second primer of a PCR primer pair is sometimes referred to herein as the "anti-sense" primer because it is derived from the anti-sense strand and it hybridizes to a sense strand of a nucleic acid. With respect to the BDV genome which is RNA, the primers of this invention listed in Table 1 are designed to amplify cDNA obtained from the genomic RNA. Accordingly, the coding sequence of the cDNA is referred to as anti-genomic or positive sense as are the primers that are derived therefrom. Similarly, the primers derived from the complementary strand are referred to as genomic or negative sense.

Preferred primers pairs for amplifying BDV p24, p16, p56, p40 and the catalytic domain of L polymerase are shown in the Examples.

The PCR reaction is performed by mixing the PCR primer pair, preferably a predetermined amount thereof, with the nucleic acids of the sample, preferably a predetermined amount thereof, in a PCR buffer to form a PCR reaction admixture. The admixture is thermocycled for a number of cycles, which is typically predetermined, sufficient for the formation of a PCR amplification product, thereby enriching the sample to be assayed for BDV genetic material. Thus, as defined herein an amplification product of this invention results from the amplification of a BDV nucleic acid, either genomic RNA

or mRNA, by priming with synthesis of cDNA with random hexamers or with a particular antisense primer pair, respectively.

5 PCR is typically carried out by thermocycling, i.e., repeatedly increasing and decreasing the temperature of a PCR reaction admixture within a temperature range whose lower limit is about 30°C to about 70°C and whose upper limit is about 90°C to about 100°C. The increasing and decreasing can be continuous, but is preferably phasic  
10 with time periods of relative temperature stability at each of temperatures favoring polynucleotide synthesis, denaturation and hybridization.

The PCR reaction is performed using any suitable method. Generally it occurs in a buffered aqueous  
15 solution, i.e., a PCR buffer, preferably at a pH of 7-9, most preferably about 8. Preferably, a molar excess (for genomic nucleic acid, usually about 10<sup>6</sup>:1 primer:template) of the primer is admixed to the buffer containing the template strand. A large molar excess is  
20 preferred to improve the efficiency of the process.

The PCR buffer also contains the deoxyribonucleotide triphosphates (polynucleotide synthesis substrates) dATP, dCTP, dGTP, and dTTP and a polymerase, typically thermostable, all in adequate amounts for primer  
25 extension (polynucleotide synthesis) reaction. An exemplary PCR buffer comprises the following: 50 mM KCl; 10 mM Tris-HCl; pH 8.3; 1.5 mM MgCl<sub>2</sub>; 0.001% (wt/vol) gelatin, 200 μM dATP; 200 μM dTTP; 200 μM dCTP; 200 μM dGTP; and 2.5 units Thermus aquaticus DNA polymerase

(U.S. Patent No. 4,889,818) per 100 microliters of buffer.

5 The resulting solution (PCR admixture) is heated to about 90°C - 100°C for about 1 to 10 minutes, preferably from 1 to 5 minutes. After this heating period, the solution is allowed to cool to a temperature which is preferable for primer hybridization, usually 50°C to 60°C. The synthesis reaction may occur at from room temperature up to a temperature above which the polymerase (inducing agent) no longer functions efficiently. Thus, for example, if E. coli DNA polymerase I is used as inducing agent, the temperature is generally no greater than about 40°C. The thermocycling is repeated until the desired amount of PCR product is produced.

15 The inducing agent may be any compound or system which will function to accomplish the synthesis of primer extension products, including enzymes. Suitable enzymes for this purpose include, for example, E. coli DNA polymerase I, Klenow fragment of E. coli DNA polymerase I, T4 DNA polymerase, other available DNA polymerases, reverse transcriptase, and other enzymes, including heat-stable enzymes, which will facilitate combination of the nucleotides in the proper manner to form the primer extension products which are complementary to each nucleic acid strand. Examples of heat-stable enzymes include Thermus aquaticus DNA polymerase, Pyrococcus furiosus DNA polymerase, and Thermatoga maritima DNA polymerase, among others. Generally, the synthesis will

be initiated at the 3' end of each primer and proceed in the 5' direction along the template strand, until synthesis terminates, producing molecules of different lengths or of the same length. There may be inducing agents, however, which initiate synthesis at the 5' end and proceed in the above direction, using the same process as described above.

The inducing agent also may be a compound or system which will function to accomplish the synthesis of RNA primer extension products, including enzymes. The inducing agent may be a DNA-dependent RNA polymerase such as T7 RNA polymerase, T3 RNA polymerase or SP6 RNA polymerase. These polymerases produce a complementary RNA polynucleotide. The high turn over rate of the RNA polymerase amplifies the starting polynucleotide as has been described by Chamberlin et al, The Enzymes, ed. P. Boyer, PP. 87-108, Academic Press, New York (1982). Amplification systems based on transcription have been described by Gingeras et al, in PCR Protocols, A Guide to Methods and Applications, pp. 245-252, Academic Press, Inc., San Diego, CA (1990).

If the inducing agent is a DNA-dependent RNA polymerase and therefore incorporates ribonucleotide triphosphates, sufficient amounts of ATP, CTP, GTP and UTP are admixed to the primer extension reaction admixture and the resulting solution is treated as described above.

The newly synthesized strand and its complementary nucleic acid strand form a double-stranded molecule which can be used in the succeeding steps of the process.

PCR amplification methods are described in detail in U.S. Patent Nos. 4,683,192, 4,683,202, 4,800,159, and 4,965,188, and at least in several texts including "PCR Technology: Principles and Applications for DNA Amplification", H. Erlich, ed., Stockton Press, New York (1989); and "PCR Protocols: A Guide to Methods and Applications", Innis et al, eds., Academic Press, San Diego, California (1990). Exemplary PCR methods for use in this invention are described in the Examples. The human BDV amplification products are then analyzed by size determination, hybridization with BDV-specific probes or by sequencing.

In another embodiment of this invention, hybridizing comprises determining the nucleotide sequence of human BDV nucleic acids in PCR amplification products as described above. A nucleic acid sequence analysis determination is also contemplated for non-PCR amplified nucleic acid samples isolated from patients.

For either of the above embodiments, such an analysis on a selected nucleic acid sample is approached by a combination of (a) physiochemical techniques, based on the hybridization or denaturation of a probe strand plus its complementary target, and (b) enzymatic reactions with endonucleases, ligases, and polymerases. Nucleic acid can be assayed as either DNA or RNA.

For sequencing, a sequence in the template nucleic acid may be known, such as where the primer to be formed can hybridize to known conserved BDV sequences in another species and initiates primer extension into a region of nucleic acids for sequencing purposes, or where previous sequencing has determined a region of nucleotide sequence and the primer is designed to extend from the recently sequenced region into a region of unknown sequence. This latter process has been referred to a "directed sequencing" because each round of sequencing is directed by a primer designed based on the previously determined sequence.

In a further aspect of the invention, the presence of BDV nucleic acid in a subject is determined by other hybridization means.

In one approach for detecting the presence of BDV RNA or cDNA thereof in a duplex, an oligonucleotide that is hybridized in the duplex includes a label or indicating group that will render the duplex detectable. Typically such labels include radioactive atoms, chemically modified nucleotide bases, and the like.

The oligonucleotide can be labeled, i.e., operatively linked to an indicating means or group, and used to detect the presence of a specific nucleotide sequence in a target template.

Radioactive elements operatively linked to or present as part of an oligonucleotide probe (labeled oligonucleotide) provide a useful means to facilitate the detection of a duplex. A typical radioactive element is

one that produces beta ray emissions. Elements that emit beta rays, such as  $^3\text{H}$ ,  $^{12}\text{C}$ ,  $^{32}\text{P}$  and  $^{35}\text{S}$  represent a class of beta ray emission-producing radioactive element labels.

A radioactive oligonucleotide probe is typically prepared by enzymatic incorporation of radioactively labeled nucleotides into a nucleic acid using kinase.

Alternatives to radioactively labeled oligonucleotides are oligonucleotides that are chemically modified to contain metal complexing agents, biotin-containing groups, fluorescent compounds, and the like.

One useful metal complexing agent is a lanthanide chelate formed by a lanthanide and an aromatic beta-diketone, the lanthanide being bound to the nucleic acid or oligonucleotide via a chelate forming compound such as an EDTA-analogue so that a fluorescent lanthanide complex is formed. See U.S. Patent No. 4,374,120, No. 4,569,790 and published Patent Application Nos. EP0139675 and WO87/02708.

Biotin or acridine ester-labeled oligonucleotides and their use to label polynucleotides have been described. See U.S. Patent No. 4,707,404, published Patent Application EP0212951 and European Patent No. 0087636. Useful fluorescent marker compounds include fluorescein, rhodamine, Texas Red, NBD and the like.

A labeled oligonucleotide present in a duplex renders the duplex itself labeled and therefore distinguishable over other nucleic acids present in a sample to be assayed. Detecting the presence of the label in the duplex and thereby the presence of the

duplex, typically involves separating the duplex from any labeled oligonucleotide probe that is not hybridized to a duplex.

5 Techniques for the separation of single stranded oligonucleotide, such as non-hybridized labeled oligonucleotide probe, from duplex are well known, and typically involve the separation of single stranded from double stranded nucleic acids on the basis of their chemical properties. More often separation techniques  
10 involve the use of a heterogeneous hybridization format in which the non-hybridized probe is separated, typically by washing, from the duplex that is bound to an insoluble matrix. Exemplary is the Southern blot technique, in which the matrix is a nitrocellulose sheet and the label  
15 is  $^{32}\text{P}$  (Southern, J. Mol. Biol., 98:503, 1975).

The oligonucleotides can also be advantageously linked, typically at or near their 5'-terminus, to a solid matrix, i.e., aqueous insoluble solid support. Useful solid matrices are well known in the art and  
20 include cross-linked dextran such as that available under the tradename SEPHADEX from Pharmacia Fine Chemicals (Piscataway, NJ); agarose, polystyrene or latex beads about 1 micron to about 5 mm in diameter, polyvinyl chloride, polystyrene, cross-linked polyacrylamide,  
25 nitrocellulose or nylon-based webs such as sheets, strips, paddles, plates microtiter plate wells and the like.

It is also possible to add "linking" nucleotides to the 5' or 3' end of the member oligonucleotide, and use



the linking oligonucleotide to operatively link the member to the solid support.

5 In nucleotide hybridizing assays, the hybridization reaction mixture is maintained in the contemplated method under hybridizing conditions for a time period sufficient for the oligonucleotides having complementarity to the predetermined sequence on the template to hybridize to complementary nucleic acid sequences present in the template to form a hybridization product, i.e., a complex  
10 containing oligonucleotide and target nucleic acid.

The term hybridizing and phrase "hybridizing conditions" and their grammatical equivalents, when used with a maintenance time period, indicates subjecting the hybridization reaction admixture, in the context of the  
15 concentrations of reactants and accompanying reagents in the admixture, to time, temperature and pH conditions sufficient to allow one or more oligonucleotides to anneal with the target sequence, to form a nucleic acid duplex. Such time, temperature and pH conditions  
20 required to accomplish hybridization depend, as is well known in the art, on the length of the oligonucleotide to be hybridized, the degree of complementarity between the oligonucleotide and the target, the guanidine and cytosine content of the oligonucleotide, the stringency  
25 of hybridization desired, and the presence of salts or additional reagents in the hybridization reaction admixture as may affect the kinetics of hybridization. Methods for optimizing hybridization conditions for a

given hybridization reaction admixture are well known in the art.

Hybridization can be carried out in a homogeneous or heterogeneous format as is well known. The homogeneous hybridization reaction occurs entirely in solution, in which both the oligonucleotide and the nucleic acid sequences to be hybridized (target) are present in soluble forms in solution. A heterogeneous reaction involves the use of a matrix that is insoluble in the reaction medium to which either the oligonucleotide, polynucleotide probe or target nucleic acid is bound.

Where the nucleic acid containing a target sequence is in a double-stranded (ds) form, it is preferred to first denature the nucleic acid, as by heating or alkali treatment, prior to conducting the hybridization reaction. The denaturation of the nucleic acid can be carried out prior to admixture with a oligonucleotide to be hybridized, or can be carried out after the admixture of the nucleic acid with the oligonucleotide.

Predetermined complementarity between the oligonucleotide and the template is achieved in two alternative manners. A sequence in the template nucleic acid may be known, such as where the primer to be formed can hybridize to conserved regions in other BDV species sequences and initiates primer extension into a region of nucleic acid for subsequent assaying purposes as described herein, or where previous sequencing has determined a region of nucleotide sequence and the primer

is designed to extend from the recently sequenced region into a region of unknown sequence.

Effective amounts of the oligonucleotide present in the hybridization reaction admixture are generally well known and are typically expressed in terms of molar ratios between the oligonucleotide to be hybridized and the template. Preferred ratios are hybridization reaction mixtures containing equimolar amounts of the target sequence and the oligonucleotide. As is well known, deviations from equal molarity will produce hybridization reaction products, although at lower efficiency. Thus, although ratios where one component can be in as much as 100-fold molar excess relative to the other component, excesses of less than 50-fold, preferably less than 10-fold, and more preferably less the 2-fold are desirable in practicing the invention.

One hybridization means contemplated for use herein is detection of membrane-immobilized BDV target sequences. In the DNA (Southern) blot technique, specific regions of cDNA are detected by immobilizing the target sequences on a membrane. The specific regions of cDNA are prepared by either PCR amplification, by PCR amplification followed by digestion with restriction endonucleases or by digestion with a restriction endonuclease without PCR amplification. Genomic RNA is first isolated. Specific regions of the genomic RNA are then PCR amplified to generate target sequences that are then analyzed intact or subjected to restriction digestion.

The above-generated target sequences are then separated according to size in an agarose gel and transferred (blotted) onto a nitrocellulose or nylon membrane support. Conventional electrophoresis separates fragments ranging from 100 to 30,000 base pairs while pulsed field gel electrophoresis resolves fragments up to 20 million base pairs in length. The location on the membrane containing a particular target sequence is then determined by direct visualization of stained target. In other aspects, the sequence migration is determined by hybridization with a specific, labeled nucleic acid probe.

In alternative embodiments, target sequences are directly immobilized onto a solid-matrix (nitrocellulose membrane) using a dot-blot (slot-blot) apparatus, and analyzed by probe-hybridization. See U.S. Patents No. 4,582,789 and No. 4,617,261.

Immobilized target sequences may be analyzed by probing with allele-specific oligonucleotide (ASO) probes, which are synthetic oligomers of approximately 20 nucleotides, preferably 17 nucleotides in length. These probes are long enough to represent unique sequences in the genome, but sufficiently short to be destabilized by an internal mismatch in their hybridization to a target molecule. Thus, any sequences differing at single nucleotides may be distinguished by the different denaturation behaviors of hybrids between the ASO probe and normal or mutant targets under carefully controlled hybridization conditions.

Alternatively, the target sequences may be detected in solution. Several rapid techniques that do not require nucleic acid purification or immobilization have been developed. For example, probe/target hybrids may be  
5 selectively isolated on a solid matrix, such as hydroxylapatite, which preferentially binds double-stranded nucleic acids. Alternatively, probe nucleic acids may be immobilized on a solid support and used to capture target sequences from solution. Detection of the  
10 target sequences can be accomplished with the aid of a second, labeled probe that is either displaced from the support by the target sequence in a competition-type assay or joined to the support via the bridging action of the target sequence in a sandwich-type format.

15 In the oligonucleotide ligation assay (OLA), a ligase is used to covalently join two synthetic oligonucleotide sequences selected so that they can base pair with a target sequence in exact head-to-tail juxtaposition. Ligation of the two oligomers is  
20 prevented by the presence of mismatched nucleotides at the junction region. This procedure allows for the distinction between known sequence variants in samples of cells without the need for purification. The joint of the two oligonucleotides may be monitored by immobilizing  
25 one of the two oligonucleotides and observing whether the second, labeled oligonucleotide is also captured.

In a further embodiment, nucleotide substitutions in BDV sequences are detected by scanning techniques. Three techniques permit the analysis of probe/target duplexes

several hundred base pairs in length for unknown single-nucleotide substitutions or other sequence differences.

In the ribonuclease (RNase) A technique, the enzyme cleaves a labeled RNA probe at positions where it is

5 mismatched to a target RNA or DNA sequence. The

fragments may be separated according to size and the approximate position of the mutation identified. See

U.S. Patent No. 4,946,773.

In the denaturing gradient gel technique, a probe-

10 target duplex is analyzed by electrophoresis in a

denaturing gradient of increasing strength. Denaturation

is accompanied by a decrease in migration rate. A duplex

with a mismatched base pair denatures more rapidly than a perfectly matched duplex.

15 A third method relies on chemical cleavage of

mismatched base pairs. A mismatch between T and C, G, or

T, as well as mismatches between C and T, A, or C, can be

detected in heteroduplexes. Reaction with osmium

tetroxide (T and C mismatches) or hydroxylamine (C

20 mismatches) followed by treatment with piperidine cleaves

the probe at the appropriate mismatch.

## 2. Detecting Human BDV Antigens and Antibodies

25 The human BDV polypeptides and antibodies of the present invention respectively described in Sections D and E are useful in various diagnostic applications for detecting BDV, BDV antigens and antibodies thereto in a sample. In preferred aspects,

the sample is from a human subject suspected of being infected with BDV. Along with detection of BDV nucleic acids as described above, the detection of human BDV antigens and/or antibodies thereby allows for an

5 additional means for diagnosis of a subject positive for BDV infection, that may or not be made concurrent with other forms of diagnosis, such as behavioral analyses.

Preferred immunoassay methods for use in BDV antigen or antibody include liquid phase immunoassays, immunoblot  
10 analyses such as Western blot, competitive and noncompetitive protein binding assays, enzyme-linked immunosorbant assays (ELISA), immunofluorescence analysis including flow cytometry, and others commonly used and widely described in scientific and patent literature, and  
15 many employed commercially. All of these assays are well known to one of ordinary skill in the art. An exemplary ELISA assay for detecting BDV is described in International Publication WO96/21020, the disclosures of which are hereby incorporated by reference.

20 Thus, in one embodiment, a method for detecting a BDV antigen in a sample comprises the steps of:

(a) contacting a sample with an anti-human BDV polypeptide antibody of this invention, such as an anti-human BDV p24 polypeptide antibody comprising antibody  
25 molecules that immunoreact with human BDV and a p24 polypeptide comprising an amino acid residue sequence selected from the group consisting of SEQ ID NO 20, SEQ ID NO 21, SEQ ID NO 22, MATGPSSLVDSLEDEEDP (SEQ ID NO 32) and RIYPQLPSAPTADEWDIIP (SEQ ID NO 33) for a time period

sufficient to allow the antibody to immunoreact with the BDV antigen present in the sample thereby forming an antigen:antibody complex; and

(b) detecting the BDV antigen in the antigen:antibody complex.

Although described as exemplary, the method described above is not limited to a BDV p24 polypeptide but rather the method encompasses and extends to all the BDV compositions of this invention as described in Sections B, D and E.

As used herein, a sample includes cells and fluid samples obtained from a subject, preferably human, who is suspected of having BDV as previously defined. Such samples thus contain BDV antigen that includes BDV viral proteins, polypeptides and fragments thereof. A cellular body sample comprises any cell that is infectable by BDV. Preferred cell samples are PBMC as discussed previously. Preferred fluid samples include any that are suspected of containing BDV and BDV antigens including polypeptides. Such physiologic fluid samples are blood, plasma, serum, urine, cerebrospinal fluid, saliva and the like.

The BDV antibodies for use in the assay method are those described in Section E that recognize human BDV and specific polypeptides thereof. The specific remaining human BDV polypeptides including p16, p56, p40 and the catalytic domain polypeptides are as described in Section D and are incorporated herein for use in defining anti-BDV polypeptide immunoreactivity. For some aspects of detecting BDV antigen, the BDV antibodies are labeled



directly. Alternatively, the assay methods are performed with the addition of a detecting antibody that binds to the anti-human BDV polypeptide antibody that is labeled. Preferred labels include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds and bioluminescent compounds. Labeling aspects are described further in Section G. In some aspects, the methods are preferably performed with the anti-human BDV polypeptide antibody immobilized on a solid support.

The term "contacting" refers to any means by which a body sample is exposed to an anti-human BDV polypeptide antibody, including admixing, adding, and the like, that allows for the BDV antigen in the sample to immunoreact with or bind specifically to the provided antibody. The resultant admixture is then maintained for a time period sufficient for the BDV antigen to come in contact with and bind to the antibody under immunoreaction conditions for a predetermined time period such as about 10 minutes to about 16-20 hours at a temperature of about 4°C to about 45°C, such time being sufficient to allow formation of an antigen:antibody complex also referred to as an immunocomplex or immunoreaction product.

Immunoreaction assay conditions are those that maintain the biological activity of the immunochemical reagents of this invention and the BDV antigen sought to be assayed. Those conditions include a temperature range of about 4°C to about 45°C, a pH value range of about 5 to about 9 and an ionic strength varying from that of

distilled water to that of about one molar sodium chloride. Methods for optimizing such conditions are well known in the art.

Those skilled in the art will understand that there  
5 are numerous well known clinical diagnostic chemistry procedures in which an immunochemical reagent of this invention can be used to form an antigen:antibody complex. Thus, various heterogenous and homogenous protocols, either competitive or noncompetitive, can be  
10 employed in performing an assay method of this invention.

Also contemplated is a method for detecting a BDV ligand in a sample, where the preferred BDV ligand is an antibody but is not so limited as it can include receptors, other soluble ligands and the like. A  
15 preferred method comprises the steps of:

(a) contacting a sample with a human BDV polypeptide of this invention, such as a substantially purified polypeptide corresponding to human Borna disease virus (BDV) p24 polypeptide comprising an amino acid  
20 residue sequence selected from the group consisting of SEQ ID NO 20, SEQ ID NO 21, SEQ ID NO 22, MATGPSSLVDSLEDEEDP (SEQ ID NO 32) and RIYPQLPSAPTADEWDIIP (SEQ ID NO 33), for a time period sufficient to allow the polypeptide to immunoreact with the BDV antibody thereby  
25 forming an immunoreaction complex; and

(b) detecting the BDV antibody in the immunoreaction complex.

As with the methods to detect BDV antigen, the detection of BDV ligand in a sample, preferably serum, is

obtained through the formation of a complex containing the BDV polypeptide and corresponding ligand. The complex is also referred to as an immunoreaction complex. The remaining human BDV polypeptides of this invention including p16, p56, p40 and the catalytic domain polypeptide, as previously defined in Section D, are similarly contemplated for use in the above method.

This approach also may utilize detection means by the addition of a detecting antibody that binds to the BDV antibody in the body sample. The detecting antibody thus contains a label that provides for detection in methods as described above and also including the indirect immunofluorescence focus assay.

#### G. Diagnostic Kits

Another aspect of the present invention is a diagnostic kit for use in detecting the presence of BDV nucleic acid, polypeptide or ligand thereof, preferably a BDV antibody, in a subject by analyzing a physiological sample from that subject. It will be readily appreciated that the presence of BDV is ascertained with kits providing means for detecting the BDV genome, BDV-specific antibodies, and BDV antigens.

A first type of kit is supplied for use with the subject nucleic acids as described in Section C and F1. Thus, the human BDV nucleic acids as previously described are thus used as probes and primers for detection, by hybridization means, of the BDV genome, transcripts thereof including cDNA, are provided in a kit. As

previously described, hybridizing means preferably includes PCR and sequencing.

The kit comprises one or more containers comprising separate containers having a human BDV nucleic acid  
5 corresponding to human BDV p24, p16, p56, p40 and the catalytic domain polypeptide, for which the nucleic acids and encoded polypeptides have been previously defined in Sections C and D, and are therefore incorporated into this section for support thereof.

10 The kits of this invention comprise a packaging means in which the reagents to practice the method are contained. A packaging means can be any type of a container in which reagents can be secured. The term "package" refers to a solid matrix or material such as  
15 glass, plastic (e.g., polyethylene, polypropylene and polycarbonate), paper, foil and the like capable of holding within fixed limits a diagnostic reagent such as a polynucleotide of the present invention. Thus, for example, a package can be a bottle, vial, plastic and  
20 plastic-foil laminated envelope or the like container used to contain a contemplated diagnostic reagent.

"Instructions for use" of the packaged reagent are also typically included and include a tangible expression describing the reagent concentration or at least one  
25 assay method parameter such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, use of control polynucleotide sequences, temperature, buffer conditions and the like.

In one aspect of providing a diagnostic kit, the nucleic acids may be labeled with a detectable label. Radioactive elements are useful labeling agents and may be useful herein. An exemplary radiolabeling agent is a  
5 radioactive element that produces alpha ray emissions. Elements which themselves emit alpha rays, such as  $^{32}\text{P}$ ,  $^{35}\text{S}$ , and  $^{33}\text{P}$  represent one class of alpha ray emission-producing radioactive element indicating groups. Particularly preferred is  $^{32}\text{P}$ . Also useful is a beta  
10 emitter, such as  $^{111}\text{In}$  or  $^3\text{H}$ .

The reagent species, polynucleotide or amplifying agent of any diagnostic system described herein can be provided in solution, as a liquid dispersion or as a substantially dry power, e.g., in lyophilized form.

15 The packaging materials discussed herein in relation to diagnostic systems are those customarily utilized in diagnostic systems.

The materials for use in the assay of this invention are ideally suited for the preparation of a kit having  
20 sufficient amounts of materials to perform at least one assay.

Additional types of kits are also supplied for use in this invention for the detection of BDV antigens and antibodies respectively with the subject anti-BDV-  
25 polypeptide specific antibodies and BDV polypeptides as described above. Thus, the diagnostic kit or system includes, in an amount sufficient to perform at least one assay, a subject BDV polypeptide and/or a subject antibody or monoclonal antibody of the present invention,

as a separately packaged reagent. Exemplary diagnostic methods for detecting BDV in a body sample and utilizing a BDV polypeptide or antibody of this invention are described in Section F.

5        In another embodiment, a diagnostic system, preferably in kit form, is contemplated for assaying for the presence of a BVD polypeptide or anti-BVD antibody in a body fluid sample such as for monitoring the fate of therapeutically administered BVD polypeptide or anti-BVD  
10       antibody. The system includes, in an amount sufficient for at least one assay, a subject BVD polypeptide and/or a subject antibody as a separately packaged immunochemical reagent. Such kits may also comprise positive control samples containing either BDV polypeptides  
15       and antibodies thereto and/or negative controls lacking such reagents.

         Frequently, it will be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may be present in from about 1 to 99%  
20       weight of total composition. Where a second antibody capable of binding to either a provided BDV anti-polypeptide antibody or to a sample antibody is employed in an assay, this will usually be present in a separate container. The second antibody is typically conjugated  
25       with a label as described below and formulated in an analagous manner to that of provided BDV polypeptides and antibodies.

         Instructions for use of the packaged reagent(s) are also typically included in the kits for detecting BDV

antigen or antibody. Such instructions generally include a tangible expression describing the reagent concentration or at least one assay method parameter such as the relative amounts of reagent and sample to be  
5 admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions and the like.

As used herein, the term "package" refers to a solid matrix or material such as glass, plastic (e.g., polyethylene, polypropylene or polycarbonate), paper,  
10 foil and the like capable of holding within fixed limits a polypeptide, polyclonal antibody or monoclonal antibody of the present invention. Thus, for example, a package can be a glass vial used to contain milligram quantities of a contemplated polypeptide or antibody or it can be a  
15 microtiter plate well to which microgram quantities of a contemplated polypeptide or antibody have been operatively affixed, i.e., linked so as to be capable of being immunologically bound by an antibody or antigen, respectively.

20 A diagnostic system of the present invention preferably also includes a label or indicating means capable of signaling the formation of an immunocomplex containing a polypeptide or antibody molecule of the present invention.

25 As used herein and as described in Section F, the terms "label" and "indicating means" in their various grammatical forms refer to single atoms and molecules that are either directly or indirectly involved in the production of a detectable signal to indicate the

presence of a complex. Any label or indicating means can be linked to or incorporated in an expressed protein, polypeptide, or antibody molecule that is part of an antibody or monoclonal antibody composition of the present invention, or used separately, and those atoms or molecules can be used alone or in conjunction with additional reagents. Such labels are themselves well-known in clinical diagnostic chemistry and constitute a part of this invention only insofar as they are utilized with otherwise novel proteins methods and/or systems.

The labeling means can be a fluorescent labeling agent that chemically binds to antibodies or antigens without denaturing them to form a fluorochrome (dye) that is a useful immunofluorescent tracer. Suitable fluorescent labeling agents are fluorochromes such as fluorescein isocyanate (FIC), fluorescein isothiocyanate (FITC), 5-dimethylamine-1-naphthalenesulfonyl chloride (DANSC), tetramethylrhodamine isothiocyanate (TRITC), lissamine, rhodamine 8200 sulphonyl chloride (RB 200 SC) and the like. A description of immunofluorescence analysis techniques is found in DeLuca, "Immunofluorescence Analysis", in Antibody As a Tool, Marchalonis, et al., eds., John Wiley & Sons, Ltd., pp. 189-231 (1982), which is incorporated herein by reference.

In preferred embodiments, the indicating group is an enzyme, such as horseradish peroxidase (HRP), glucose oxidase, or the like. In such cases where the principal indicating group is an enzyme such as HRP or glucose



oxidase, additional reagents are required to visualize the fact that a complex (immunoreactant) has formed.

Such additional reagents for HRP include hydrogen peroxide and an oxidation dye precursor such as

5 diaminobenzidine. An additional reagent useful with glucose oxidase is 2,2'-amino-di-(3-ethyl-benzthiazoline-G-sulfonic acid) (ABTS).

Radioactive elements are also useful labeling agents and are used illustratively herein. An exemplary

10 radiolabeling agent is a radioactive element that produces gamma ray emissions. Elements which themselves emit gamma rays, such as  $^{124}\text{I}$ ,  $^{125}\text{I}$ ,  $^{128}\text{I}$ ,  $^{132}\text{I}$  and  $^{51}\text{Cr}$  represent one class of gamma ray emission-producing radioactive element indicating groups. Particularly

15 preferred is  $^{125}\text{I}$ . Another group of useful labeling means are those elements such as  $^{11}\text{C}$ ,  $^{18}\text{F}$ ,  $^{15}\text{O}$  and  $^{13}\text{N}$  which themselves emit positrons. The positrons so emitted produce gamma rays upon encounters with electrons present in the animal's body. Also useful is a beta emitter, 20 such  $^{111}\text{In}$  indium or  $^3\text{H}$ .

The linking of labels, i.e., labeling of, polypeptides and proteins is well known in the art. For instance, antibody molecules produced by a hybridoma can be labeled by metabolic incorporation of radioisotope- 25 containing amino acids provided as a component in the culture medium. See, for example, Galfre et al., Meth. Enzymol., 73:3-46 (1981). The techniques of protein conjugation or coupling through activated functional groups are particularly applicable. See, for example,

Aurameas, et al., Scand. J. Immunol., Vol. 8 Suppl. 7:7-23 (1978), Rodwell et al., Biotech., 3:889-894 (1984), and U.S. Pat. No. 4,493,795.

5 If desired, a reagent can be typically affixed to a solid matrix by adsorption from an aqueous medium although other modes of affixation applicable to proteins and polypeptides can be used that are well known to those skilled in the art. Exemplary adsorption methods are described herein.

10 Useful solid matrices are also well known in the art. Such materials are water insoluble and include the cross-linked dextran available under the trademark SEPHADEX from Pharmacia Fine Chemicals (Piscataway, NJ); agarose; beads of polystyrene beads about 1 micron ( $\mu$ ) to  
15 about 5 millimeters (mm) in diameter available from Abbott Laboratories of North Chicago, IL; polyvinyl chloride, polystyrene, cross-linked polyacrylamide, nitrocellulose- or nylon-based webs such as sheets, strips or paddles; or tubes, plates or the wells of a  
20 microtiter plate such as those made from polystyrene or polyvinylchloride.

25 The reagent species or amplifying reagent of any diagnostic system described herein can be provided in solution, as a liquid dispersion or as a substantially dry power, e.g., in lyophilized form. Where the indicating means is an enzyme, the enzyme's substrate can also be provided in a separate package of a system. A solid support such as the before-described microtiter plate and one or more buffers can also be included as

separately packaged elements in this diagnostic assay system. The packaging materials discussed herein in relation to diagnostic systems are those customarily utilized in diagnostic systems.

5

### Examples

The following examples are intended to illustrate but are not to be construed as limiting of the specification and claims in any way.

10

#### 1. Isolation of Human BDV

##### A. Patient Profile

Coded serial blood samples from 33 randomly selected psychiatric patients, taken during acute disease episodes, and from twenty healthy blood donors were analyzed in a double-blind manner for the expression of BDV antigens in peripheral blood mononuclear cells

15

(PBMC). Results from these studies led to the selection of three patients designated H1, H2, and H3, to attempt the isolation and molecular characterization of human BDV. These particular patients were selected based on their relative high numbers of positive PBMC for BDV antigens in serially collected blood samples. Patients were selected from two follow-up cohort studies with psychiatric patients in Berlin (Bode et al., Nature Med., 1:232-236 (1995); Bode, Curr. Top. Microbiol. Immunol., 190:101-128 (1995)).

20

25

Patients H1 and H3 with an acute mental disorder were from the Psychiatric Department, Benjamin Franklin

Hospital, Free University of Berlin, Germany. Samples and clinical diagnoses were given by R. Ferszt; additional psychological evaluation was conducted by E. Severus. Patient H2 with a chronic mental illness was from the Psychiatric Department, Theodor-Wenzel-Werk and District Hospital Berlin-Zehlendorf (Heckeshorn), Germany. Samples and clinical records were given by W. Schwalbe, and further comments on the clinic history provided by G. Arkenberg and E.H. Kang-Welberts.

Clinical diagnoses were according to DSM-III-R criteria (American Psychiatric Association, 1987).

H1 is a 45-year-old female patient with a Bipolar Disorder mixed with psychotic features (DSM-III-R : 296.64). H2 is a 37-year-old male patient suffering from a chronic Obsessive Compulsive Disorder (DSM-III-R : 300.30; 305.00), who has been hospitalized for the last fourteen years. H3 is a 55-year-old male patient with a history of a Bipolar disorder (DSM-III-R : 296.70).

#### B. Isolation of Human BDV from Patients

Preliminary studies done using ultrasonically disrupted BDV antigen-positive patients' PBMC to inoculate young rabbit brain cells failed to allow BDV growth. In addition, previously reported attempts to recover BDV using cerebrospinal fluid from BDV seropositive patients to infect fetal rabbit brain cells were also unsuccessful (Rott et al., Arch. Virol., 118:143-149 (1991)). Therefore, an alternative approach was adopted based on the co-cultivation of patients' PBMC

with BDV-infectable cells such as a human oligodendroglial cell line, previously documented to be highly sensitive to BDV infection (Briese et al., Proc. Natl. Acad. Sci., USA, 89:11486-11489 (1992)).

5        Samples of PBMC from healthy control individuals were obtained from blood donors officially registered with the Federal Health Office (BGA), Germany (Bundesgesundhbl, 31:286 (1988)).

10        Blood samples of 9 ml from each of the control and patient subjects were collected at the hospital in the presence of sodium citrate (10 mM final concentration) as anti-coagulant. Samples were coded and separated into plasma and PBMCs by centrifugation on Ficoll-hypaque. PBMCs were processed for detection of virus antigen and  
15        BDV RNA in a double-blind manner.

For virus isolation, PBMC samples isolated within eight hours after blood sample collection were then transported from the site of collection in the hospital to a tissue culture facility where BDV-infected material  
20        had never been previously used. For co-cultivation experiments, PBMC ( $1 \times 10^5$ ) were resuspended in DMEM containing 10% FCS and added to  $1 \times 10^5$  cells of a human oligodendroglial (OL) cell line previously documented to be highly sensitive to BDV. For isolation of human BDV,  
25        PBMCs were used not later than 12 hours after their isolation from blood samples. Every three days the cells were subcultured at a 1:4 dilution. Passages were done by disaggregation of the cells using trypsin treatment.

Expression of BDV antigen was examined every three passages using immunofluorescence procedures.

Isolation of BDV required least 10 passages of co-cultured OL cells. All co-cultivation studies were  
5 conducted in a double-blind manner.

After the twelvth passage, OL cells co-cultured with PBMC obtained from patients H1, H2 or H3 expressed high levels of BDV antigens. Infectivity titers associated with BDV-infected cell were determined using a focus  
10 forming unit (FFU) assay. For this assay, BDV-infected OL cells ( $1 \times 10^7$ ) were resuspended in 1 ml of DMEM containing 1% FCS and ultrasonicated. Infectivity was determined on young rabbit brain cell monolayers. Virus yields was in the range of 5 to  $10 \times 10^6$  FFU/ $1 \times 10^7$  OL  
15 cells.

As shown herein and below, three patients with mood disorders have been shown to contain infectious BDV. BDV isolation required long-term co-cultivation (ten or more passages) of the patients' PBMCs with OL cells, a cell  
20 line highly susceptible to BDV infection. Virus isolation also required the use in co-cultivation experiments of multiple serially collected PBMCs samples from each corresponding patient, specifically, ten, three, and ten serial samples for patients H1, H2 and H3,  
25 respectively. Successful virus isolation appeared to correlate with the use for the co-cultivation experiments of PBMCs samples expressing the highest levels of virus antigens and RNA.

2. Detection and Characterization of BDV RNA in Patients

A. PCR Amplification of BDV Nucleic Acid Fragments

Polymerase chain reaction (PCR) amplification  
5 of BDV nucleic acid was performed on RNA isolated from  
PBMC or from OL that had been co-cultured with PBMC as  
described above. The particular PCR procedure used was  
reverse-transcriptase PCR (RT-PCR) in which total RNA  
isolated from infected cells is first reverse transcribed  
10 into cDNA that is then used as the template for PCR. PCR  
primers are thus designed to hybridize to the cDNA  
template for detecting various regions of BDV genome.

Amplification of BDV RNA from the PBMC preparation  
as described below documented BDV infection of human  
15 patients. The detection of BDV RNA in OL confirmed the  
infectivity of BDV presence in patients suspected of  
having BDV. From either PBMC or OL cell preparations,  
total RNA was extracted from the cells using the acid  
guanidinium thiocyanate-phenol chloroform method  
20 described by Chomczynski et al., Anal. Biochem., 162:156-  
159 (1987)). Purified RNA (0.5 to 2.0 µg) was reverse  
transcribed to form cDNA using 50 U of Moloney murine  
leukemia virus reverse transcriptase (GIBCO BRL,  
Gaithersburg, MD) and random hexamers (2.5 mM) as primers  
25 (Krug et al., Methods Enzymol., 152:316 (1987)).

As a first determination of the presence of BDV in  
humans, PBMC were screened for the presence of BDV p24  
nucleotide sequences by amplifying cDNAs prepared above  
using BDV-specific primers 2.1 and 2.2 as listed in

Tables 1 and 2. The position of the primers as well as others used to amplify various BDV polypeptide-encoding fragments of this invention are shown in Figure 1. In the figure, a diagram of the entire BDV genome RNA (anti-genomic polarity) and relative location of primers are shown. The genome organization presented in the diagram is based on the complete sequence of two BDV RNA genomes as yet reported, strain V and C6BV (Briese et al., Proc. Natl. Acad. Sci., USA, 89:11486-11489 (1994); Cubitt et al., J. Virol., 68:1382-1396 (1994); de la Torre, J. Virol., 68:7669-7675 (1994)). The GenBank Accession Numbers for strain V and C6BV RNA genome sequences are U04608 and L27077, respectively.

The consensus nucleotide sequence, presented in compliance with the IUPAC-IUB Biochemical Nomenclature Commission (referred to herein as IUPAC) code and derived from the sequences reported by Cubitt et al. and Briese et al., is shown in Figure 2A-Figure 2H (SEQ ID NO 1). Figures 2A-2H also show the Cubitt et al. and Briese et al. sequences respectively labeled BDV JCT and BDV Briese. Dots in the BDV JCT and BDV Briese sequences indicate that both sequences are consistent with each other and thus comprise the consensus sequence shown in Figures 2A-2H and in SEQ ID NO 1. Nucleotide differences at specific nucleotide positions are shown for the BDV JCT and BDV Briese sequences. The substitutions as shown are then indicated in the consensus sequence in accordance with the IUPAC code.



The BDV-specific primers designated 2.1 and 2.2 for amplifying p24 were designed to allow for the amplification of the full length open reading frame (ORF) encoding the BDV p24 polypeptide. Table 1 indicates the following aspects: the designation of the primer, the nucleotide sequence listed in 5' to 3' direction, and the designated SEQ ID NO. The sequences and nucleotide positions of the p24 primers as well as the others described herein are based on the C6BV genome RNA sequence (Cubitt et al., J. Virol., 68:1382-1396 (1994); de la Torre, J. Virol., 68:7669-7675 (1994)). The accession number for this sequence is L27077. Table 2 contains the polarity of the DNA primers based on the negative polarity of the BDV RNA genome and the nucleotide positions (nt) of the primers corresponding to the intact BDV RNA negative polarity genome based on the DNA sequence, presented in the anti-genomic polarity, as shown in Figure 1.

Table 1

<u>Primer</u>	<u>Nucleotide Sequence*</u>	<u>SEQ ID NO</u>
2.1	CAGGAGGCTCAATGGCAACG	42
2.2	TTTATGGTATGATGTCCCAC	43
3.1	ATCGAATCACCATGAATTCAAAGC	44
3.2	GTCAGTATTGCAACTAAGGC	45
4.4	GCACGCAATTAATGCAGC	46
3030R	CAGTGTAGGCCTAAGCTTGTG	47
2962F	AAGTTGAGAAGGCGGCGTAG	48
4.3	CGGTACGGTTTATTCTCTGC	49

	5248F	TGACCATGAGCTCAACGGC	50
	5936R	GCATGATGATGTTAAGCAGGC	51
	BV259F	TTCATACAGTAACGCCCAGC	52
	BV829R	GCAACTACAGGGATTGTAAGGG	53
5	BV277F	GCCTTGTGTTTCTATGTTTGC	54
	BV805R	GCATCCATACATTCTGCGAG	55

\* The nucleotide sequences are written in the 5' to 3' direction.

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Table 2

	<u>Primer</u>	<u>Polarity</u>	<u>Nucleotide Positions in</u> <u>BDV RNA Genome</u>
	2.1	Anti-genomic	1261-1280
	2.2	Genomic	1859-1879
15	3.1	Anti-genomic	1882-1905
	3.2	Genomic	2315-2334
	4.4	Anti-genomic	2225-2242
	3030R	Genomic	3010-3030
	2962F	Anti-genomic	2962-2981
20	4.3	Genomic	3738-3756
	5248F	Anti-genomic	5248-5267
	5936R	Genomic	5915-5936
	BV259F	Anti-genomic	259-278
	BV829R	Genomic	808-829
25	BV277F	Anti-genomic	277-297
	BV805R	Genomic	786-805

The conditions for PCR amplification were as follows: 94°C for 5 min (1 cycle); 94°C for 1 min, 55°C

for 1 min, 72°C for 1 min (35 cycles); 72°C extension for 10 min (1 cycle). Each PCR reaction used 1/10 of the cDNA product, 50 pmol of each primer, 1 U of Taq polymerase, 100 mM of each deoxynucleotide triphosphate, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 10 mM Tris-HCl (pH 9.0) and 0.1% Triton X-100 in a final volume of 50 µl.

The PBMC-amplified BDV p24 PCR products, not detectable by ethidium bromide staining, were then cloned and transformants screened with a <sup>32</sup>P probe corresponding to internal p24 as described below in Example 2. In addition, the sequence of three independent clones for p24 ORF derived from each patient's PBMCs was also determined as described below in Example 2.

As a control for RNA quality, the cDNAs were also amplified with specific primers to generate a 192 bp GAPDH fragment (Buesa-Gomez et al., J. Med. Virol., 42:193-197 (1994)). Each RT-PCR assay also included two water samples as negative controls of environmental contamination, and a BDV-negative C6 RNA sample.

For PCR amplification of BDV p40 sequences, the PBMC-derived RNA prepared as described above is used in a nested PCR procedure using 2 pairs of primers. The first PCR is done using 1/10 of the cDNA product and BDV primers BV259F (anti-genomic polarity) and BV829R (genomic polarity), shown in Tables 1 and 2, corresponding to nucleotide positions 259 to 278 and 808 to 829, respectively, in the BDV genome as shown in Figures 2A-2B. The amplification step is performed as described above. The products of the first reaction are

then used as the template for the second reaction which generates a smaller product, the sequence of which is nested within the first product. The second PCR is done using 1/25 of the RT-PCR product and the nested set of  
5 BDV primers: BV277F (anti-genomic polarity) and BV805R (genomic polarity), corresponding to nucleotide positions 277 to 297 and 786 to 805, respectively, in the BDV genome. Amplification is done using the same cycle conditions as in the first PCR. The set of nested  
10 primers used amplifies a final 528 bp segment of BDV p40. The BDV specificity of the RT-PCR products is then determined by Southern blot hybridization using a <sup>32</sup>P-labeled probe corresponding to internal sequences of the BDV p40 528 bp segment.

15 To unequivocally identify the isolated infectious agent as BDV, and determine its relationship to the BDV sequences directly derived from PBMCs, as well as to previously known BDV genomic sequences, RNA was extracted from OL cells co-cultivated with patients' PBMCs as  
20 described above. RNA was reverse transcribed and the corresponding cDNAs subjected to PCR as described below using pairs of primers to amplify three BDV open reading frames (ORFs), corresponding to the p24, p16, and p56 BDV polypeptides, as well as the putative catalytic domain of  
25 the BDV L polymerase. RT-PCR was followed by cloning and sequencing of the amplified human BDV products. BDV p40 is also amplified from the OL cells as described above for PBMC.

Full length p24 ORF having 603 nt including 15 additional nt of primer sequences outside the ORF and a 426 nt p16 ORF were amplified by PCR using primer pairs 2.1+2.2, and 3.1+3.2, respectively (Table 1 and Figure 1). Amplification of full length p56 ORF having 1509 bp was done using two pairs of primers, 4.4+3030R and 2962F+4.3 (Table 1 and Figure 1), that generated two overlapping PCR fragments with sizes of 805 bp and 794 bp, respectively, that covered the entire BDV p56 ORF. The putative catalytic domain of BDV L polymerase (de la Torre, J. Virol., 68:7669-7675 (1994)) having a fragment size of 688 nt was amplified using primers BV 5248F and BV 5936R (Table 1 and Figure 1). PCR primers specific for the human BDV isolates of this invention are readily made based on the sequence information presented in Example 2 and are used in PCR as described herein to amplify patient BDV.

In all cases PCR products were readily detected by gel electrophoresis following ethidium bromide staining of the gel. In control assays, however, RT-PCR assays done with RNA extracted from OL cells co-cultivated with BDV-antigen negative PBMC samples from healthy control individuals failed to amplify any BDV-specific product in several independent experiments.

#### B. Detection of Human BDV PCR Products

##### 1) Molecular Characterization of Human BDV Isolates

To confirm the identify of the 618 bp PCR amplified BDV p24 ORF fragments resulting from amplification with the 2.1 and 2.2 primer pairs as described above, the resultant fragments were detected by Southern blot hybridization using a <sup>32</sup>P-probe corresponding to an internal p24 fragment (nucleotides 1329 to 1749 in the BDV RNA genome as shown in Figures 2C and 2D and SEQ ID NO 1).

The results of the Southern blot are shown in Figure 3. The control sample, labeled C in lane 1, is PBMC-isolated RNA, subjected to RT-PCR, from a representative healthy control individual negative for BDV antigen; in lanes 2-4, PBMC-isolated RNA was run from psychiatric patients H1, H2, and H3, respectively. The figure corresponds to a composite of the autoradiographic segment on the top showing the results of southern blot hybridization and the part of the gel on the bottom showing the ethidium bromide staining of GAPDH amplified fragment in the control as well as the patient samples. Track M corresponds to the 1 kb ladder DNA (GIBCO BRL, Gaithersburg, MD). The top and bottom of the composite were lined up with respect to the migration of the 1 kb ladder DNA (track M).

Treatment of RNA samples with DNase-free RNase, but not with RNase-free DNase prior to the RT-PCR assay, as well as the omission of the reverse transcriptase enzyme, prevented amplification of both BDV p24 and GAPDH sequences.

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adapted to its culture environment. For this purpose, RNA was directly isolated from a single plaque of LCMV Armstrong strain, clone 5 3b. This plaque was grown in Vero cells and originated from a LCMV population that had  
 5 been serially passaged thirty times in Vero cells using for each passage a multiplicity of infection of 0.1 plaque forming units (PFU)/cell. RNA was reversed transcribed using random hexamers as described above and PCR conducted using a pair of primers to specifically  
 10 amplify a 362 bp fragment of the LCMV glycoprotein following procedures described by Evans et al., J. Virol., 68:7367-7373 (1994). The estimated mutation frequency found was less than  $2.5 \times 10^{-4}$  substitutions per nucleotide for Taq polymerase errors, indicating that it  
 15 is very unlikely that Taq polymerase errors are responsible for the differences found between human BDV isolates and BDV strain V as described below.

Figures 4A-4C show the respective nucleotide sequence alignment of ORFs p24 (4A), p16 (4B) and p56  
 20 (4C) among the human BDV isolates (H1, H2, and H3), C6BV and BDV strain V. Dots in each of the figures indicate the same nucleotide as the one found for that position in the BDV strain V sequence. Numbers on the right correspond to last nucleotide position of each row within the corresponding ORF. The complete RNA genome sequences  
 25 of strain V and C6BV have been published as previously described and have the accession numbers U04608 and L27077, respectively. Corresponding to p24 nucleotide sequences shown in Figure 4A, the strain V, H1, H2 and H3



sequences are respectively listed as SEQ ID NO 2, SEQ ID NO 3, SEQ ID NO 4 and SEQ ID NO 5. The p24 polypeptides encoded by full-length ORF p24 in the last three SEQ ID NOs are respectively listed in SEQ ID NO 20 (Figure 8A),  
5 SEQ ID NO 21 (Figure 8B) and SEQ ID NO 22 (Figure 8C).

Similarly, for the p16 nucleotide sequences shown in Figure 4B, the strain V, H1, H2 and H3 sequences are respectively listed as SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8 and SEQ ID NO 9. The p16 polypeptides encoded by  
10 full-length p16 ORF in the last three SEQ ID NOs are respectively listed in SEQ ID NO 23 (Figure 9A), SEQ ID NO 24 (Figure 9B) and SEQ ID NO 25 (Figure 9C).

Moreover, for the p56 nucleotide sequences shown in Figure 4C, the strain V is listed as SEQ ID NO 10. The  
15 p56 nucleotide sequence for patient H1 is listed as SEQ ID NO 11. As the p56 nucleotide sequences are identical among patients H2 and H3, only one nucleotide sequence is presented for both of them as SEQ ID NO 12. Accordingly, the encoded amino acid residue sequences for patients H1  
20 and H2/H3 are respectively listed in SEQ ID NOs 26 (Figure 10A) and 27 (Figure 10B). The respective sequences for C6BV strain are not listed as SEQ ID NO.

Partial p40 nucleotide sequences having 571 nt for  
25 patients H1, H2 and H3 are shown in Figures 5A-1 and 5A-2 as compared to the corresponding regions in C6BV and Strain V, along with a consensus sequence. The nucleotide alignments are as previously described. The 571 nt region corresponds to the region amplified by the

first primer pair indicated above while a 528 nt region results from nested PCR as previously described. The p40 consensus sequence is listed as SEQ ID NO 13. The H1, H2 and H3 patient sequences are respectively listed as SEQ ID NO 13, SEQ ID NO 14 and SEQ ID NO 15. The corresponding respective encoded human BDV p40 full-length polypeptides are listed in SEQ ID NO 28 (Figure 11A), SEQ ID NO 29 (Figure 11B) and SEQ ID NO 30 (Figure 11C).

Partial catalytic domain of the L polymerase nucleotide sequences having 689 nt for patients H1, H2 and H3 are shown in Figures 5B-1 through 5B-3 as compared to the corresponding regions in C6BV and Strain V. A consensus sequence for this domain is also shown and is listed as SEQ ID NO 17. Patient H1 and H2 sequences are identical and listed as SEQ ID NO 18 while H3 having one nucleotide difference is listed as SEQ ID NO 19. However, the nucleotide difference does not result in an alteration of encoded amino acids. Thus, all three patients have the same encoded catalytic domain encoded polypeptide sequence listed in SEQ ID NO 31 (Figure 31).

Direct cloning and sequencing of BDV ORFs p24 and p16 present in the patients' PBMC revealed that the p16 sequence determined directly from RNA isolated from PBMC of each of the three patients (H1, H2, and H3), was identical to the corresponding p16 sequences obtained from RNA isolated from OL cells after co-cultivation with patient PBMC. p24 sequences determined prior and after co-cultivation of patients' PBMC with OL cells were also

identical in patients H1 and H2, whereas in the case of patient H3, one single nucleotide silent change (C->T) was found in the third base position of codon 127.

OL cells co-cultivated with PBMCs from patients H1, H2, and H3, but not from healthy controls, expressed BDV-specific RNAs. Sequence analysis of RT-PCR products obtained using RNA from OL cells co-cultivated with patients' PBMCs and specific primers to amplify BDV ORFs p24, p16, p56, and the catalytic domain of the L polymerase, unequivocally identified these isolates as human BDV.

The three human BDV isolates showed a high degree of sequence conservation with respect to BDV strain V and C6BV genome sequences, as well as BDV sequences determined in samples from naturally infected animals of different species. BDV strain V and C6BV sequences had more than 95% homology at the nucleotide level (Briese et al., Proc. Natl. Acad. Sci., USA, 91:4382-4386 (1994); Cubitt et al., J. Virol., 68:1382-1396 (1994)), which is remarkably high for two RNA virus isolates with different origin and passage history (Holland, Curr. Top. Microbiol. Immunol., 176 (1992); Morse, ed. "The Evolutionary Biology of Viruses", Raven, New York (1994)). Both viruses, BDV strain V and C6BV were originally isolated from two different naturally infected horse brains and passed several times in rabbits, followed by passages in rats (Schneider et al., J. Virol., 68:63-68 (1994)). In addition, both viruses were maintained as a persistent infection for more than twenty

passages either in OL cells, in the case of BDV strain V, or in the rat astrocytoma C6 cells for C6BV, before their corresponding genome sequences were determined.

Each of the three human BDV isolates of this invention had an unique sequence, differing from the other two at one or two nucleotide positions in each of the ORFs analyzed here, with the exception of identical p56 sequences found for H2 and H3 (Figure 4C) and the catalytic domain for H1 and H2 (Figures 5B-1 through 5B-3), where in the latter, the nucleotide change was present in the 3' primer sequence. Levels of divergence between the human BDV isolates and C6BV at the nucleotide level were similar to those found for p24 sequences between BDV isolates from horses separated by more than ten years and with different history of passages in tissue culture. Moreover, high nucleotide sequence conservation, with only 0.3% divergency, has been also reported among p24 BDV sequences from naturally infected horses and sheep (Binz et al., Virus Res., 34:281-289 (1994)). The human BDV isolates displayed a high level of sequence conservation with respect to BDV strain V, with divergencies of 0.5% to 0.83% for p24, 0.23% to 0.47% for p16, and 0.07% to 0.20% for p56 (Figure 6B as futher described below). In addition, cloning and sequencing of the segment of BDV ORF V corresponding to the putative catalytic domain of the viral L polymerase, revealed a complete amino acid conservation between the three human BDV isolates and BDV strain V not including the region corresponding to the 3' primer.

No insertions or deletions were observed in p24, p16 and p56 ORFs sequences of the human BDV isolates compared to the corresponding reported sequences for BDV strain V and C6BV. When compared to C6BV, most of the nucleotide changes in human BDV isolates were transition events, accounting for 92.8 to 93.7%, 93.7 to 94.1%, and 87 to 88% of the substitutions found in p24, p16, and p56, respectively. Mutations in p24, p16 and p56 ORFs were randomly distributed with not apparent regions of higher variability.

The nature and total number of amino substitutions, as well as total number of nucleotide differences, in ORFs p24, p16 and p56 among the human BDV isolates and the horse-derived strain V and C6BV isolates are summarized in Figure 6A and Figure 6B.

In Figure 6A, the amino acid differences found in ORFs p24, p16 and p56 among the three human BDV isolates (H1, H2, and H3), C6BV and strain V are presented. Amino acids are presented in the single letter code. Numbers on top correspond to the codon position within each ORF. The dashes indicate correspondence to the C6BV amino acid at the particular amino acid positions. Figure 6B shows a triangular matrix summarizing the total number of nucleotide (upper right) and amino acid (lower left) substitutions among the BDV human isolates (H1, H2, and H3), C6BV and strain V.

All three human BDV isolates had the substitution Ala->Thr at amino acid position 326 in p56 compared with C6BV and BDV strain. The H1 isolate also had two amino

acids, Thr and Leu at positions 412 and 501, respectively, in p56, that are not found in C6BV or BDV strain V sequences. Isolates H1 and H3 had Glu at position 4 in p24, an amino acid not previously seen at this position in any of the BDV p24 sequences as yet reported in other species. In addition, isolate H1 had Thr at position 127 in p24, instead of the His found in all the other BDV p24 sequences as yet determined.

The possibility that the OL cells became BDV positive as result of a contamination with BDV from laboratory sources can be excluded because of the following: 1) Co-cultivation experiments and later passages of the infected cells were conducted in a tissue culture facility where V-infected material had never been previously used; 2) PBMC samples were coded and the investigators conducting tissue culture work operated in a double-blind manner and in ten independent control experiments OL cells co-cultivated with BDV-antigen negative PBMC samples did not express BDV antigen and RNA after more than 30 passages; 3) Sequence data indicated that each of the three human BDV isolates are genetically more closely related to BDV strain V than C6BV and RT-PCR assays, cloning of amplified products and sequence determination were conducted where BDV strain V had not been previously handled; 4) Changes in p24 and p16 BDV strain V sequences were not found after more than twenty passages in OL cells. This finding likely reflects the adaptation in the laboratory of BDV strain V to grow in OL cells. In contrast, each of the human BDV isolates

recovered by co-cultivation of patients' PBMC with OL cells, differed from the other two BDV human isolates and from BDV strain V in their p24 and p16 sequences (Figure 4A and 4B); and 5) Direct cloning and sequencing of BDV ORFs p24 and p16 present in the patients' PBMC, without amplification through co-cultivation with OL cells revealed that p24 and p16 sequences present in PBMC of patients H1 and H2 were identical to the p24 and p16 sequences determined after co-cultivation of the corresponding PBMC with OL cells. In the case of patient H3, comparison of sequences derived directly from PBMC and after co-cultivation with OL cells showed no differences in p16 and one single silent nucleotide change in p24, corresponding to the transition C->T in the third base of codon 127. This result also suggests sequence stability during BDV replication in OL cells.

Because the mutation frequencies of RNA viruses exceed, by more than a millionfold, those of their eukaryotic hosts, extremely rapid virus evolution is anticipated and frequently observed. However, RNA viruses can also exhibit long-term stasis both in nature and in laboratory experiments as result of selection for fit master sequences in rather constant environments. Although only limited sequence information is presently available, the data presented herein for the first time indicate a high level of sequence conservation among BDV isolates and BDV polypeptide-encoding regions from PBMC of the three psychiatric patients and from naturally infected field animals of different species. This

finding suggests that BDV strain V-like sequences may represent prevalent and stable species of BDV in nature, with the ability to infect a number of different animal species including humans. It is worth noting that frequently one single, or very few, amino acid changes can cause drastic phenotypic changes in RNA viruses, including altered tropism and pathogenicity. Furthermore, the host's genetics also contributes to disease phenotypes caused by BDV infection.

This invention thus provides direct evidence of BDV infection in humans and a method for screening for such as described herein for both nucleic acids and proteins including BDV antigens and antibodies.

#### C. Analysis of BDV mRNA

BDV persistent infection in a variety of cultured cells is characterized by the absence of cytolysis and lack of cell-free virus production, but relative high levels of viral RNA transcription and replication (de la Torre et al., Virology, 179:853-856 (1990)). Northern blot hybridization studies revealed that RNA isolated from OL cells co-cultivated with the PBMC of patients H1, H2, and H3 displayed the expected pattern of BDV-specific transcripts detectable with a BDV p24 probe (Cubitt et al., J. Virology, 68:1382-1396 (1994) as shown in Figure 7.

For the Northern analysis, total RNA (10 µg) from each sample was analyzed by hybridization using specific probes for BDV p24 and GAPDH as respectively shown in the



upper and middle portions of the figure. Lanes 1 and 5 correspond to RNA from OL cells infected with PBMC from two representative healthy control individuals negative for BDV antigen; lanes 2-4, correspond to RNA from OL cells co-cultured with PBMC from patients H1, H2, and H3, respectively. RNA from C6 (lane 6) and C6BV (lane 7) cells, were used as negative and positive controls, respectively, of BDV hybridization. The lower part of the figure shows the ethidium bromide staining of the RNA gel.

OL cells co-cultivated with PBMC from healthy control volunteers expressed neither viral antigens nor BDV-specific RNAs after more than twenty passages as shown in lanes 1 and 5. These results indicated that infectious BDV, with the ability to replicate in OL cells, was present in PBMC from patients H1, H2, and H3.

#### D. Detection of Patient-Derived BDV Antibodies

##### 1) Preparation of Synthetic BDV Peptides

The synthetic BDV peptides used in practicing the methods of this invention are synthesized using standard solid-phase synthesis techniques as, for example, described by Merrifield, Adv. Enzymol., 32:221-296 (1969), and Fields, G.B. and Noble, R.L., Int. J. Peptide Protein Res., 35:161-214 (1990) or for example, by the simultaneous multiple peptide synthesis method using the solid-phase technique described by Houghten, Proc. Natl. Acad. Sci. USA, 82:5131-5135 (1985). The synthesized peptides are then analyzed by reverse phase

high performance liquid chromatography (HPLC) on a Vydac C-18 column (Alltech Associates, Inc., IL) with a 0-60% acetonitrile linear gradient in 0.1% trifluoroacetic acid. Peptides are then purified to homogeneity by preparative HPCL using optimal conditions suggested by the analytical chromatography. Amino acid compositions and concentrations of isolated peptides are determined by subjection to 24 hour hydrolysis in 6 N HCl in evacuated tubes at 110 degrees Celsius (110°C) and subsequent analysis on a Beckman Model 6300 High Performance Analyzer.

Purified peptides are separately resuspended in distilled water to form a dissolved peptide solution.

The amino acid residue sequences of BDV peptides listed in Table 3 are derived from the amino acid sequences of BDV p40, p24, p16 and p56 antigens. The selection of BDV peptide sequences, as well as the immunization of animals using these peptides, is as described in "Peptides as Immunogens", in Current Topics in Microbiology and Immunology, Vol. 130, Koprowski and Melcher (eds), Springer-Verlag, Berlin, Heidelberg (1986).

Table 3

<u>Peptide Designation</u>	<u>SEO ID NO</u>	<u>Amino Acid Sequence*</u>
p24-1	32	MATGPSSLVDSLEDEEDP
p24-2	33	RIYPQLPSAPTADEWDIIP
p16-1	34	MNSKHSYVELKGKVIVPG
p16-2	35	RLRNIGVGPLGPDIRSSGP
p56-1	36	GLSCNTDSTPGLIDLEIR

	p56-2	37	RSKLRRRRRDTQQIEYLV
	p56-3	38	LISLCVSLPASFARRRRRLGRWQE
	p40-1	39	MPPKRRLVDDADAMEDQD
	p40-2	40	MEDQDDLYEPPASLPKLP
5	p40-3	41	ELSGEISAIMRMIGVTGLV

\* Amino acid sequence written in single letter code

The resultant peptides are then used in the methods of this invention to determine the presence of BDV antibody in a subject as well as be used as immunogens to raise anti-peptide polyclonal and monoclonal antibodies as described below.

## 2) Preparation of Recombinant BDV Peptides

BDV p40, p24 and p16 recombinant peptides were expressed as trpE-fusion proteins in E. coli BL21/DE3 cells (Studier and Moffat, J. Mol. Biol., 189:113-130 (1986), for use in the pATH vector system (Korner et al., Methods Enzymol., 194:477-490 (1991)). An amino-terminal 826 bp fragment of the BDV p40 gene was excised with XbaI/HindIII from the pCRII vector containing the subcloned p40 gene, referred to as pCRII-p40, prepared as described in Example 2B. The excised p40 gene was then ligated into a similarly digested pATH2 vector (ATCC Accession Number 37696). The complete p24 ORF was excised with EcoRI from the pCRII vector containing the p24 ORF as prepared in Example 2B. The resultant p24 gene was then ligated into a similarly digested pATH1 vector (ATCC Accession Number 37695).

XmaI-digested PCR-amplified DNA corresponding to full length p16 ORF was inserted into a similarly digested pATH2 vector.

A p56-encoding fragment corresponding to full-length p56 cDNA was obtained by PCR using primers BV2225F (5'CGAATTCGCACGCAATTAATGCAGC3' (SEQ ID NO 56) and BV3738R (5'GCTACTCGAGCGGTACGGTTTATTCCTGC3' (SEQ ID NO 57). With the exception that restriction cloning sites for facilitating directional cloning into the multiple cloning site of the pCRII vector rather than using the TA cloning region were used with the above primer pairs, the resulting amplified fragment was cloned into pCRII as previously described. In addition, a truncated form of p56 cDNA was prepared to generate as a trpE fusion protein in the pATH2 vector system where the p56 portion begins at a methionine residue located at amino acid residue position 150 of the p56 full-length protein. To generate the truncated cDNA, 5' and 3' primers were used in PCR having the respective sequences

5'CCCCCGGGCAATGTACTGCAGTTTCGCGGACT3' (SEQ ID NO 58) and 5'GGGCCCCGGGTTATTCCTGCCACCGGCCGA3' (SEQ ID NO 59).

The resultant pATH vectors containing BDV genes were then used in the bacterial expression system for generating trpE recombinant BDV fusion proteins, the expression of which was performed as described by Korner et al., Methods Enzymol., 194:477-490 (1991). Protein extracts from bacteria expressing the trpE-BDV fusion proteins were then separated by SDS-PAGE. The

electrophoresed recombinant proteins were then recovered from the gel using 50 mM  $\text{NH}_4\text{HCO}_3$ , 3%  $\beta$ -mercaptoethanol as an elution buffer. Recombinant catalytic domain proteins are similarly prepared as described above.

In an alternative approach to generate recombinant BDV proteins for use in the methods of this invention, BDV genes were amplified by PCR from the respective cDNAs for use in a Baculovirus expression vector system. The 5' and 3' primer pairs used in amplifying p40 had the respective nucleotide sequences

5'ATGCCACCCGGGAGACGCCTGATTGAT3' (SEQ ID NO 60) and 5'CGGATCCCGGGCTAGTTTAGACCAGTCACTCC3' (SEQ ID NO 61). The 5' and 3' primer pairs used in amplifying p24 had the respective nucleotide sequences

5'GGCCATATGCGCCCGGGCCCATCGAGTCTGGTCGACTCCCTG3' (SEQ ID NO 62) and 5'CTCGAGCCCGGGTTATGGTATGATGTCCCACTCATC3' (SEQ ID NO 63). The 5' and 3' primer pairs used in amplifying p16 have the respective nucleotide sequences

5'CGAATCCCGGGGAATTCAAAGCATTCTTA3' (SEQ ID NO 64) and 5'TCCCCCGGGCAGTATTGCAACTAACGG3' (SEQ ID NO 65).

Amplification procedures of p56 and the catalytic domain are similarly performed.

Underlined nucleotides in the above primers indicate BDV specific sequences. The resultant amplified DNA fragments were then digested with XmaI and separately ligated into similarly digested Baculovirus transfer vectors, pAC409 (p40 and p16) and pAC401 (p24) (Summers and Smith, "A Manual of Methods for Baculovirus and

Insect Cell Culture Procedures", Texas Agricultural  
Experimental Station, Bulletin 1555, College Station,  
Texas.

Recombinant baculoviruses were then generated by  
5 cotransfection of SF158 (Spodoptera frugiperda) insect  
cells with the respective recombinant baculovirus transfer  
vectors and linearized BaculoGold DNA (Pharmingen, La  
Jolla, CA) as a source of baculovirus DNA using  
lipofection as described by Groebe et al., Nuc. Acids  
10 Res., 18:4033 (1990). SF158 cells were maintained in  
TC100 medium supplemented with penicillin (40 IU/ml) and  
streptomycin (50 µg/ml). The expression of the different  
recombinant proteins in insect cells was verified by  
Western blot using polyclonal rabbit antisera for each  
15 protein that is prepared as described below.

The resultant recombinant BDV proteins were then  
used as immunogens to prepare anti-BDV antibodies (see  
Example 2E) and are separately used in assays to allow  
the detection of BDV antigens in an individual as  
20 described below.

### 3) Detection of Patient-Derived BDV Antibodies

With the synthetic BDV peptides as  
25 described above, BDV antibodies in sera from an  
individual are detected with immunological based methods,  
including ELISA as described in International Publication  
WO/21020, the disclosures of which are hereby

incorporated by reference, Western blot, and the like procedures.

BDV antibodies present in an individual are also detected by using the purified recombinant BDV bacterial-expressed proteins prepared above or with lysates of the recombinant BDV eucaryotic-expressed (insect cells). For either, the proteins are separated by 12.5 or 15% SDS-PAGE performed as described by Grasser et al., J. Virol., 65:3779-3788 (1991). After electrophoretic transfer onto a nitrocellulose membrane, the membrane is preincubated at 25°C for 30 minutes in blocking solution (5% nonfat dry milk in PBS). The blocked membrane is then exposed overnight at 4°C to selected test sera diluted 1:20 in blocking solution or with rabbit antisera diluted 1:100 (also used for testing antibody titers and specificity). Membranes are then washed three times in PBS, incubated for 1 hour at 25°C with the appropriate secondary antibody labeled with horseradish peroxidase and diluted 1:500 in blocking solution. After washing three times with PBS, bound antibodies are visualized with diaminobenzidine in hydrogen peroxide or by the enhanced chemiluminescence method with the ECL kit (Amersham Buchler, Braunschweig, Germany).

For competition experiments, approximately  $10^7$  baculovirus-infected SF158 cells are washed twice with ice-cold PBS and resuspended in 1 ml of lysis buffer containing 100 mM Tris-HCl, pH 9.0, 100 mM NaCl, 5 mM KCl, 0.5 mM  $MgCl_2$ , 1 mM  $CaCl_2$ , 1 mM DTT, 0.5% Triton X-100, 1 mM PMSF and 50  $\mu$ M leupeptin. After 15 minutes on

ice, debris is pelleted and 300  $\mu$ l of clarified supernatant is added to 100  $\mu$ l of test sera and maintained for 8-10 hours at 4°C prior to adding to the electrophoresed BDV proteins for processing as described above.

E. Analysis of BDV Antigens

1) Preparation of Polyclonal Antisera to Synthetic and Recombinant Polypeptides

For preparation of a peptide immunogen, the synthetic and recombinant polypeptides are prepared as described above.

The proteins (25-50  $\mu$ g) are then separately emulsified in 1 ml of PBS and 1 ml of complete or incomplete Freund's adjuvant (DIFCO Laboratories, Detroit, MI) to prepare immunogens. For each BDV protein, two rabbits as well as two rats are immunized by intradermal injection and subsequently boosted subcutaneously at three-week intervals with 25-50  $\mu$ g of immunogen in Freund's incomplete adjuvant. Rats are used as rat anti-BDV antibodies have been known to have lower background of non-specificity. Moreover, rat spleens from immunoresponsive rats are then used to generate monoclonal antibodies as described below.

Blood is then collected three times during week 3 and 14 and the serum analyzed for the presence of BDV-specific antibodies by Western blot or by ELISA performed as described by Budleston et al., Aids Res. Human Retrovirus, 9:939-944 (1993) or in International



Publication WO/21020. Further specificity and antibody-binding characteristics are then assessed with immunofluorescence analyses and immunoprecipitation procedures using COS-7 cells transfected with the  
5 corresponding BDV ORF under control of a cytomegalovirus immediate early promoter (pRc/CMV-BDV). With the latter system, good levels of expression of BDV ORFs as determined with immunofluorescence has been detected with serum from a BDV-infected rat.

10 Anti-p56 antibodies to truncated p56 recombinant protein prepared as described in Example 2D were obtained with the above-described methods for use in the present invention.

Immunospecific peptide antibodies are then further  
15 purified by ammonium-sulfate precipitation (0-45%), followed by purification of IgG on an ion-exchange Mono Q column (Pharmacia LKB, Piscataway, NJ) connected to a fast protein liquid chromatography (FPLC) system (Pharmacia). Purified anti-peptide polyclonal antibodies  
20 are then used in assays for detecting BDV antigens in patient samples as described below.

## 2) Preparation of Monoclonal Antibodies

The peptide and recombinant protein  
25 immunogens prepared above are also used to generate monoclonal antibodies in rats. The immunogens are separately injected into rats as described above with a boost of 50  $\mu$ g of the prepared peptide intravenously

(i.v.) in normal saline 4 days prior to fusion and a second similar perfusion boost one day later.

The animals so treated are sacrificed and the spleen of each mouse is harvested. A spleen cell suspension is then prepared from which spleen cells are isolated and used to prepare hybridomas according to the methods well known to one of ordinary skill in the art.

The resultant anti-BDV peptide hybridomas are then screened for immunoreactivity as described above.

Selected positive clones are then subcloned and used for detecting BDV antigens in patient samples as described below.

### 3) Detection of BDV-Specific Antigens

BDV-specific antigens were detected in cells expressing BDV-specific RNAs as determined by indirect immunofluorescence as described by Cubitt et al., J. Virol., 68:1371-1381 (1994). Briefly, cells were washed once with PBS and fixed in acetone/methanol for 5 minutes at room temperature. After several washes, the fixed cells were then incubated in PBS containing 5% normal goat serum for 45 minutes at room temperature to block non-specific background. Cells were then incubated with the following antibodies: 1) BP-11, a serum from an experimentally BDV-infected splenectomized rabbit; 2) L149, a cerebrospinal fluid (CSF) sample isolated from a horse with BDV; 3) KFU2 and WI monoclonal antibodies that specifically recognize p24 and p40 BDV antigens, respectively. In other assays, the anti-peptide

antibodies of this invention along with anti-BDV p24, p16, p56, p40 and the catalytic domain recombinant proteins produced as described above, are used in the above-described method to detect BDV antigens present in PMBC isolated from suspected BDV-infected subjects.

After incubation with the primary antibodies for 60 minutes at room temperature, exposed cells are washed five times with PBS and reacted with the corresponding fluorescein isothiocyanate-conjugated secondary antibodies to allow detection of immunoreaction product.

BDV antigens are also detected by flow cytometry performed as described by Bode et al., Nature Med., 1:232-236 (1995). Briefly, patient PBMC are first fixed with paraformaldehyde (2.5% for 30 minutes) and treated with Triton X-100 (1% for 45 minutes). BDV antigens are detected with the above-described antibodies and fluorescently labeled secondary antibodies.

The foregoing specification, including the specific embodiments and examples, is intended to be illustrative of the present invention and is not to be taken as limiting. Numerous other variations and modifications can be effected without departing from the true spirit and scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: de la Torre, Juan C.

(ii) TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR SCREENING  
OF HUMAN BORNA DISEASE VIRUS

(iii) NUMBER OF SEQUENCES: 65

(iv) CORRESPONDENCE ADDRESS:

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(F) ZIP: 92037

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
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(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Fitting, Thomas  
(B) REGISTRATION NUMBER: 34,163  
(C) REFERENCE/DOCKET NUMBER: TSRI 465.0

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8910 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

	GTTGCGTTAA CAACAAACCA MTCATYATYC TTCTAACAAA ATGAACACAC GCAATGCCAC	60
10	CCAAGAGACG CCTGGTTGAT GACGCCGATG CCATGGAGGA YCAAGATYTA TATGAACCCC	120
	CAGCGAGCCT CCCYAAGCTC CCYGGRAAAT TCCTACAATA CACCGTTGGG GGGTCTGACC	180
15	CGCATCCGGG TATAGGGCAT GAGAARGAYA TCAGGCAGAA CGCAGTGGCA TTGTTAGACC	240
	AGTCACGGCG CGATATGTTT CAYACAGTAA CGCCYAGCCT TGTGTTTCTA TGTGTTGCTAA	300
	TCCCAGGACT GCACGCTGCG TTTGTTTCAGG GAGGGGTGCC TCGTGAATCY TACCTGTCGA	360
20	CGCCTGTYAC GCGTGGRGAA CAGACTGTYG TTAAGACTGC RAAGTTTTAC GGGGAAAAGA	420
	CRACRCAGCG TGATCTCACC GAGCTGGAGA TCTCCTCTAT MTCAGCCAT TGTGCTCAT	480
25	TACTAATWGG GGTGTGATA GGATCGTCRT CTAAGATYAA AGCAGGAGCC GAGCAGATCA	540
	AGAAAAGGTT TAAAACTATG ATGGCAGCCT TAAACCGGCC ATCCCATGGT GAGACTGCTA	600
	CACTACTYCA GATGTTTAAT CCACATGAGG CTATAGATTG GATTAACGGC CARCCCTGGG	660
30	TAGGCTCCTT TGTGTTGTCT CTACTAACTA CAGACTTTGA GTCCCCAGGT AAAGAATTYA	720
	TGGAYCAGAT TAARCTTGTC GCAAGTTATG CRCAGATGAC TACGTACACT ACTATAAAGG	780
35	AGTACCTCGC AGAATGYATG GATGCTACCC TTACAATCCC YGTAGTTGCA TATGAGATYC	840
	GTGACTTTTT AGAAGTTTCA GCAAAGCTTA ARGAGGAWCA TGCTGACCTG TTYCCGTTYC	900
	TGGGGGCIAT TMGRCACCCC GACGCTATCA AGCTKGC GCC ACGRAGCTTT CCAATCTGG	960
40	CYTCYGCAGC GTTTTACTGG AGTAAGAAGG ARAAYCCCAC AATGGCRGGC TACCGGGCCT	1020
	CCACCATCCA GCCGGGCGCR AGTGTCAAGG ARACCCAGCT TGCCCGGTAT AGGCGCCGCG	1080
45	AGATATCTCG YGGRGARGAC GGGGCAGAGC TCTCAGGTGA GATCTCTGCC ATAATGARRA	1140
	TGATAGGTGT GACTGGTCTA AACTARAAAA CAATGAACAA ACCAATAAAA AACCAAATGC	1200
	GGCAAACCCY CCGCGACCTG YGATGAGYTC CGACCTCCGG CTGACATTGC TTGAAYTAGT	1260
50	CAGGAGGCTC AATGGCAACG SGACCATCGA GTCTGGTCTA CTCCCTGGAG GACGAAGAAG	1320

	ATCCCCAGAC	ACTACGACGG	GAACGAYCGG	GGTCACCAAG	ACCACGGAAG	RTCCCAAGGA	1380
	ATGCATTGAC	CCAACCRGTA	GACCAGCTCC	TGAAGGACCT	CAGGAAGAAC	CCCTCCATGA	1440
5	TCTCAGACCC	AGACCAGCGA	ACCGGAAGGG	AGCAGCTRTC	GAATGATGAG	CTWATCAAGA	1500
	AGYTAGTGAC	GGAGCTGGCC	GAGAATAGCA	TGATCGAGGC	TGAGGAGGTG	CGGGGCACTC	1560
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10	AAACCATCCA	GACAGCTCAG	CGGTGCGAYC	ACTCCGAYAG	CATCAGRATC	CTYGGCGAGA	1680
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15	AGAAGGTGGA	YCTCCTCTAC	GCATCAACCG	CCGTTGGGAC	CTCTGCACCC	ATGTTGCCCT	1800
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20	TCAAGGRCAA	GGTAATCGTC	CCTGGATGGC	CCACACTGAT	GCTTGAGATA	GACTTTGTAG	1980
	GRGGGACTTC	ACGGAACCAG	TTCCTTAACA	TCCCATTTCT	TTCAGTGAAA	GAGCCTCTGC	2040
25	AGCTTCCACG	CGAGAAGAAG	TTGACCGACT	ACTTYACYAT	TGACGTAGAR	CCAGCAGGTC	2100
	ATTCCCTGGT	CAAYATATAC	TTCCAGATTG	ACGACTTCTT	GTCCTAACA	CTCAACTCAC	2160
	TRTCYGTRTA	CAAGGACCCG	ATTAGRAAAT	ACATGTTTCCT	ACGCCTCAAC	AAGGAMCAGA	2220
30	GCAAGCACGC	AATYAATGCA	GCYTTCAATG	TCTTYTCTTA	TGGGCTTCGG	AACATTGGTG	2280
	TTGGYCCTCT	CGGCCCRGAC	ATTGATCTT	CAGGGCCTTA	GYTGCAATAC	TGACTCCACT	2340
35	CCTGGAYTRA	TYGAYCTGGA	GATAAGGCCA	CTTTGCCACA	CCCCAACGGA	AAATGTCATT	2400
	TCATGCGAGG	TTAGTTATCT	YAACCACACG	ACTATTAGCC	TCCCGGCAGT	CCACACRTCA	2460
	TGCCTCAAGT	ACCACTGCAA	AACCTATTGG	GGATTCTTTG	GTAGCTACAG	CGCTGACCGA	2520
40	ATCATMAATC	GGTACACTGG	TACTGTTAAG	GGTTGTYTAA	ACAACTCAGC	RCCAGAGGAY	2580
	CCCTTCGAGT	GCAACTGGTT	CTACTGCTGC	TCGGCGATTA	CAACAGAGAT	CTGCCGATGC	2640
45	TCTATTACAA	ATGTCACGGT	GGCTGTRCAR	ACATTCCCAC	CGTTCATGTA	CTGCAGTTTY	2700
	GCRGACTGYA	GTACYGTGAG	YCARCAGGAG	CTAGAGAGTG	GMAAGGCAAT	GCTGAGCGAT	2760
	GGCAGTACMT	TAACTTATAC	CCCGTATATC	YTACARTCAG	AAGTCGTGAA	CAAAACCCTY	2820
50	AATGGGACYA	TACTCTGCAA	CTCATCCTCY	AAGATAGTTT	CCTTCGATGA	ATTTAGGCGT	2880

	TCATACTCCC	TARCGAATGG	TAGTTACCAG	AGCTCATCAA	TCAATGTGAC	GTGTGYAAAC	2940
	TACACGTCGT	CCTGCCGGYC	CARGTTGARA	AGGCGGCGTA	GGGAYACYCA	RCAGATTGAG	3000
5	TAYCTAGTTC	ACAAGCTTAG	GCCYACACTG	AAAGATGCRT	GGGAGGACTG	TGAGATCCTC	3060
	CAGTCTCTGC	TCCTAGGGRT	GTTTGGTACT	GGGATYGCAA	GTGCTTCKCA	ATTTYTTGAGG	3120
	RGCTGGCTCA	ACCACCCTGA	YATCATCGGG	TATATAGTTA	ATGGAGTTGG	GGTWGTCTGG	3180
10	CAATGCCATC	GTGTTAATGT	CACGTTTCATG	GCGTGGAATG	AGTCCACMTA	TTACCCTCCA	3240
	GTAGATTACA	ATGGRCGGAA	GTACTTYCTG	AATGATGAGG	GRAGGYTACA	AACAAACACC	3300
15	CCCGAGGCAA	GGCCAGGGCT	TAAGCGGGTC	ATGTGGTTCG	GCAGGTACTT	CCTAGGGACA	3360
	GTAGGGTCTG	GGGTGAAACC	GAGGAGGATT	CGGTACAATA	AGACCTCACA	TGAYTACCAY	3420
	CTRGAGGAGT	TTGAGGCAAG	TCTCAACATG	ACCCCYCAGA	CCAGTATCGC	CTCGGGTCAT	3480
20	GAGACAGACC	CCATAAATCA	TGCCTACGGA	ACGCAGGCTG	AYCTCCTTCC	ATACACCAGG	3540
	TCTAGTAATA	TAAGRCTAC	RGATACAGGC	TCAGGCTGGG	TGCACATCGG	CCTACCCTCA	3600
25	TTTGCTTTCC	TCAATCCYCT	CGGGTGGCTY	AGGGACCTAC	TTGCRGTGGG	RGCCTGGTTG	3660
	GGTGGGGTTC	TATACTTAAT	AAGTCTTTGT	GTTTCCTTAC	CAGCCTCCTT	CGCGAGGAGG	3720
	AGACGCCTCG	GCCGGTGGCA	GGAATAAACC	GTACCGACCA	RWCTCTTAAA	AACCCTCTYC	3780
30	TCGGRACAGA	GGTCTCTTTC	TGCCTTAART	CGAGYTCACT	CCCCCATCAY	GTACGAGCAY	3840
	TRGGCCAGAT	TAAAGCAARG	AACCTGGCAT	CCTGTGACTA	TTACTTGCTA	TTCCGCCAAG	3900
35	TTGTATTGCC	CCCTGAAGTA	TATCCCATTG	GTGTYTAAT	AAGAGCTGCG	GAGGCYATAC	3960
	TAACAGTTAT	AGTATCAGCT	TGGAAGCTGG	ATCAYATGAC	RAAGACCCTA	TACTCCTCTG	4020
	TGAGATATGC	ACTCACC AAT	CCCCGGGTCC	GRGCCCAACT	TGAGCTYCAC	ATTGCCTACC	4080
40	AGCGCATAGT	GGGTCAGGTC	TCGTAYAGCC	GGGARGCAGA	YATAGGGCCA	AAAAGGCTTG	4140
	GGAATATGTC	ATTGCAATTC	ATCCAATCYC	TCGTTATTGC	CACCATAGAC	ACRACRAGCT	4200
45	GCCTAATGAC	CTACAACCAC	TTTCTTGCTG	CAGCAGACAC	AGCCAAGAGC	AGATGCCAYC	4260
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	TACTAAAAAA	ACTGCTYCAA	CTTGACCCYG	TTCTCCTCCT	CATGGTCTCT	TCGGTGCAGA	4560
5	AGTCATGGTA	CTTCCCTGAG	ATCCGAATGG	TYGACGGGTC	ACGGGAGCAG	CTCCACAAGA	4620
	TGCGTGTCGA	GCTGGARACG	CCCCAAGCCC	TGCTGTCRTA	CGGCCATAACC	CTCCTGTCAA	4680
	TATTTTCGRGC	AGAGTTTATC	AAAGGCTATG	TCTCAAAGAA	TGCGAAGTGG	CGGCCYGTAC	4740
10	ACCTGCTCCC	AGGCTGTGAC	AAATCCATAA	ARAATGCGAG	AGAGCTGGGC	CGCTGGAGCC	4800
	CGGYRTTTGA	CCGACGATGG	CAGCTCTTCG	MGAAGGTTGT	CATTCTAAGA	ATTGCTGACC	4860
15	TAGATATGGA	TCCCGACTTC	AACGATATTG	TTAGCGAYAA	GGCGATAATC	AGCTCAAGAA	4920
	GGGACTGGGT	ATTYGAGTAC	AATGCAGCRG	CCTTTTGGA	GAAATACRGT	GARCGGTTGG	4980
	AGAGGCCYCC	TGCCAGRTCG	GGACCRTCAC	GRCTTG TGAA	TGCTCTRATC	GATGGACGCT	5040
20	TAGAYAATAT	CCCAGCCCTG	CTAGAGCCAT	TTTACAGGGG	AGCGGTTGAG	TTYGAGGATC	5100
	GGYTGACTGT	GCTCGTGCCT	AAGGAGAARG	AGTTRAAGGT	AAAGGGAAGG	TTCTTCTCGA	5160
25	AGCAAACATT	GGCAATCAGG	ATATATCAGG	TTGTTGCTGA	AGCTGCACTT	AAGAAYGAGG	5220
	TTATGCCATA	CYTAAARACA	CAYTCAATGA	CCATGAGCTC	AACGGCYCTA	ACYCAYCTTC	5280
	TTAACCGGCT	ATCACATACT	ATCACTAAGG	GTGACTCCTT	TGTTATTAAC	YTWGAYTATA	5340
30	GYTCCTGGTG	CAACGGTTTC	CGACCAGAAC	TRCARGCCCC	AMTCTGTGGT	CAGTTGGATC	5400
	AGATGTTCAA	TTGCGGGTAC	TTCTTCAGGA	CTGGGTGCAC	ACTGCCATGC	TTTACCACGT	5460
35	TTATTATTCA	RGACAGRTTC	AACCCGCCCT	ATTCCYTCMG	TGGTGAGCCC	GTTGAAGACG	5520
	GWGTYACATG	CGCGGTTGGG	ACTAARACAA	TGGGRGAGGG	YATGAGGCAG	AAACTATGGA	5580
	CAATYCTTAC	GAGCTGCTGG	GAGATAATTG	CTCTTCGGGA	AATTAACGTG	ACGTTTAAYA	5640
40	TACTAGGCCA	RGGTGATAAT	CAGACAATCA	TYRTACATAA	ATCTGCAAGC	CAAAATAAYC	5700
	AGCTATTAGC	GGAGCGAGCA	YTRGGRGCYY	TGTACAAGCA	TGCTAGATTA	GCTGGCCATA	5760
45	ACCTYAAGGT	AGARGAATGY	TGGGTGTCAG	ATTGTCTGTA	TGAGTATGGA	AAGAAGCTYT	5820
	TCTTCCGTGG	TGTACCTGTC	CCRGGCTGTT	TGAAGCAGCT	CTCRCGGGTG	ACGGAYTCYA	5880
	CTGGRGAGYT	ATTCCCAAAC	CTATACTCAA	AGTTAGCCTG	CTTAACATCA	TCRTGYTTAA	5940
50	GCGCAGCGAT	GGCAGACACA	TCYCCATGGG	TGGCACTCGC	GACAGGTGTC	TGTCTGTATC	6000



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	CCCTCTGYCT	CGTAGGYCCA	TCCATTGGTG	GGCTTCCRAC	YCCTGCAACC	CTRCCCAGTG	6120
5	TCTTTTTTCAG	AGGAATGTCC	GACCCAYTGC	CCTTTCAGCT	AGGACTCTTG	CAGACCCTCA	6180
	TTAARACGAC	AGGGGTGACY	TGTAGCTTGG	TGAATCGTGT	GGTYAAGTTA	CGGATAGCAC	6240
	CCTATCCAGA	CTGGCTCTCY	CTAGTGACTG	ACCCGACYTC	ACTCAACATT	GCYCARGTGT	6300
10	ACCGGCCAGA	ACGTCARATC	AGGAGGTGGA	TTGAGGARGC	RATAGCRACA	AGCTCACACT	6360
	CGTCACGCAT	AGCAACTTTY	TTCCAGCAGS	CCCTCACGGA	GATGGCYCAG	YTGCTTGCGA	6420
15	GGGACCTYTC	AACAATGATG	CCTCTTCGRC	CCCGGGATAT	GTGGGCCTTA	TTCGCATTAT	6480
	CAAATGTCCG	ATAYGGTYTA	AGCATTATAG	ATCTATTTCA	AAARTCCTCT	ACCGTTGTYT	6540
	CTGCAAGTCA	AGCTGTCCAT	ATCGARGATG	TTGCCCTAGA	GAGTGTAAGG	TATAAGGAAT	6600
20	CTATCATYCA	GGGTCTGTTA	GACACYACTG	AGGGGTAYAA	CATGCAACCT	TATTTGGAAG	6660
	GTTGCACTTA	CCTTGCAGCC	AARCAGYTAC	GKAGGTTGAC	RTGGGGTCGA	GACCTAGTTG	6720
25	GAGTYACAAT	GGCGTTTGTT	GGCGAGCAAT	TCCATCCYCA	YAGTTCTGTS	GGTGCAAARG	6780
	CRGAACTCTA	CCTCGAYGCT	ATYATATACT	GCCCACARGA	GACRTTGCGG	TCACACCATC	6840
	TGACTACCAG	GGGGGACCAG	CCGCTTTACC	TYGGATCYAA	TACGGCTGTC	AMGGTYCAGC	6900
30	GAGGTGAGAT	CACRGGCCTA	ACAAAGTCAA	GGGCTGCAAA	TCTAGTCARG	GACACTCTCG	6960
	TTCTCCAYCA	GTGGTAYAAR	GTCCGTAARG	TTACCGATCC	ACACTTGAAC	ACYCTCATGG	7020
35	CRGCTTCTT	RCTTGAGAAG	GGRTACACAT	CTGACGCTCG	RCCTAGCATY	CAGGGTGGGA	7080
	CCCTCACRCA	TCGTCTCCCA	TCCCGYGGAG	ACTCACGSCA	RGGGCTYACT	GGGTATGTRA	7140
	ATATACTMAG	YACGTGGCTY	CGRTTCTCAA	GTGATTATCT	TCACTCTTTC	TCGAAATCAT	7200
40	CAGAYGACTA	YACAATCCAC	TTYCAGCATG	TATTCACATA	CGGTTGCCTC	TATGCTGATT	7260
	CGGTGATTAG	ATCGGGCGGT	GTTATTTCCA	CTCCTTACCT	TTTGAGTGCA	AGTTGTAAAA	7320
45	CATGCTTTGA	GAAGATAGAC	TCAGAGGAGK	TCGTCTTGGC	ATGYGAACCY	CAATAYAGGG	7380
	GTGCTGAGTG	GCTGATATCA	AAGCCAGTYA	CTGTCCCTGA	GCAGATAAYT	GAYGCTGAAG	7440
	TCGAGTTTGA	CCCCTGTGTG	AGTGCGRGTT	ATTGTCTCGG	GATTCTCATT	GGCAAGTCAT	7500
50	TCTTRGTTGA	CATAAGGGCA	AGTGGGCATG	ATATYATGGA	GCAGCGGACA	TGGGCTAACY	7560

	TGGAGAGGTT TTCTGTRTCG GACATGCAGA AACTTC CRTG GAGTATTGTA ATTCGGTCTC	7620
	TCTGGAGATT CCTTATTGGC GCACGRCTCC TYCAGTTTGA GAAGGCTGGC CTYATTAGRA	7680
5	TGCTGTATGC TGCRA CAGGT CCAACCYTA GCTTCCTAAT GAAAGTYTTT CAAGACTCAG	7740
	CCCTMCTYAT GGA CTGCGCA CCYCTYGATC GGCTGTMCCC TAGGATCAAC TTTCATAGTC	7800
	GGGGAGACCT CGTYG CYAAG CTYGT TTTAT TRCCCTTCAT CAACCCGGGT ATAGTGGAGA	7860
10	TTGAAGTGTC TRGAATTAAT AGCAAGTAYC ATGCAGTATC GGAGGCYAAT ATGGATCTGT	7920
	ACATCGCTGC TGCMAARTCT GTGGGCGTRA AGCCACACACA GTTTGTTGAG GAAACAAACG	7980
15	ACTTTACGGC CCGCGGCCAC CACCATGGTT GTTATTCCTT TTCTTGGTCT AAGTCACGCA	8040
	ATCAATCACA GGTCTAAAG ATGGTAGTRC GGAAGCTGAA GCTMTGTGTC CTGTATATAT	8100
	ACCCACAGT CGATCCCGCC GTTGCTCTCG ACCTGTGCCA YCTRCCAGCA YTA ACTATAA	8160
20	TCCTAGTGCT CGGCGGTGAC CCAGCGTACT AYGAGCGATT ACTTGAGATG GACCTRTGCG	8220
	GGGCTGTGTC AAGTCGMGTY GATATCCCCC ATTCYCTRGC TGSCAGAACG CACAGGGGGT	8280
25	TCRCARTRGG CCCAGACGCT GGTCCAGGTG TRATTAGACT YGACARGTTA GAGTCRGTTT	8340
	GTTAYG CYCA CCCCTGTTTR GAGGARCTAG AGTTTAATGC RTAYCTAGAC TCTGAGTTRG	8400
	TTGAYATTAG TGATA TGTGC TGCCTCCCCY TAGCGACACC CTGTAAGGCC CTWTT CAGGC	8460
30	CARTRTATCG GAGCTTACAG TCGTTCAGGT TAGCCTTAAT GGACAACTAT AGTTTTGTMA	8520
	TGGACCTCAT TAYGATCCGR GGRSTGGACA TYAGGCCTCA CCTTGAGGAR TTTGAYGARC	8580
35	TGCTTG TGGT RGGRCAGCAY ATCCTCGGYC AGCCCGTCCT AGTRGAGGTT GTTTACTACG	8640
	TTGGAGTTGT TRGGAAGCGY CCTGTGTTAG CGAGGCATCC STGGTCAGCA GATCTTAAGC	8700
	GAATYACTGT RGGGGGGCGR GCKCCCTGCC CYTCTGCTGC YRGAYTCCGT GATGAGGATT	8760
40	GTCRGGGGTC TCTGYTGGTT GGGCTTCCYG CTGGRTTGAC GCAGTTRITG RTRRTTGATT	8820
	RAGRTYRAGC CAYCTACTRC CCTATTCTTA AAAAACCATA YGTCAGTGGT GCAGTGCTTG	8880
45	GGYTTGGTTG TTGCTTTGTT GTAGCGCKTT	8910

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

50 (A) LENGTH: 603 base pairs  
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATGGCAACGC	GACCATCGAG	TCTGGTCGAC	TCCCTGGAGG	ACGAAGAAGA	TCCCCAGACA	60
CTACGACGGG	AACGACCGGG	GTACCAAGA	CCACGGAAGG	TCCCAAGGAA	TGCATTGACC	120
CAACCAGTAG	ACCAGCTCCT	GAAGGACCTC	AGGAAGAACC	CCTCCATGAT	CTCAGACCCA	180
GACCAGCGAA	CCGGAAGGGA	GCAGCTGTCT	AATGATGAGC	TAATCAAGAA	GTTAGTGACG	240
GAGCTGGCCG	AGAATAGCAT	GATCGAGGCT	GAGGAGGTGC	GGGGCACTCT	TGGAGACATC	300
TCGGCTCGTA	TCGAGGCAGG	GTTTGAGTCC	CTGTCCGCCC	TCCAAGTGGA	AACCATCCAG	360
ACAGCTCAGC	GGTGCGATCA	CTCCGACAGC	ATCAGGATCC	TCGGCGAGAA	CATCAAGATA	420
CTAGATCGCT	CCATGAAGAC	AATGATGGAG	ACAATGAAGC	TCATGATGGA	GAAGGTGGAT	480
CTCCTCTACG	CATCAACCGC	CGTTGGGACC	TCTGCACCCA	TGTTGCCCTC	CCATCCTGCA	540
CCTCCGCGCA	TTTATCCCCA	GCTCCCAAGT	GCCCCGACAA	CGGATGAATG	GGACATCATA	600
CCA						603

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 603 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

	ATGGCAACGG AACCATCGAG TCTGGTCGAC TCCCTGGAGG ACGAAGAAGA TCCCCAGACA	60
5	CTACGACGGG AACGATCGGG GTCACCAAGA CCACGGAAGG TCCCAAGGAA TGCATTGACC	120
	CAACCAGTAG ACCAGCTCCT GAAGGACCTC AGGAAGAACC CCTCCATGAT CTCAGACCCA	180
	GACCAGCGAA CCGGAAGGGA GCAGCTGTCT AATGATGAGC TAATCAAGAA GTTAGTGACG	240
10	GAGCTGGCCG AGAATAGCAT GATCGAGGCT GAGGAGGTGC GGGGCACTCT TGGAGACATC	300
	TCGGCTCGTA TCGAGGCAGG GTTTGAGTCC CTGTCCGCCC TCCAAGTGA AACCATCCAG	360
15	ACAGCTCAGC GGTGCGATTA CTCCGACAGC ATCAGGATCC TCGGCGAGAA CATCAAGATA	420
	CTAGATCGCT CCATGAAGAC AATGATGGAG ACAATGAAGC TCATGATGGA GAAGGTGGAT	480
	CTCCTCTACG CATCAACCGC CGTTGGGACC TCTGCACCCA TGTTGCCCTC CCATCCTGCA	540
20	CCTCCGCGCA TTTATCCCCA GCTCCCAAGT GCGCCGACAA CGGATGAGTG GGACATCATA	600
	CCA	603

25 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

	(A) LENGTH: 603 base pairs
	(B) TYPE: nucleic acid
30	(C) STRANDEDNESS: single
	(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

35 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

	ATGGCAACGG GACCATCGAG TCTGGTCGAC TCCCTGGAGG ACGAAGAAGA TCCCCAGACA	60
45	CTACGACGGG AACGATCGGG GTCACCAAGA CCACGGAAGG TCCCAAGGAA TGCATTGACC	120
	CAACCAGTAG ACCAGCTCCT GAAGGACCTC AGGAAGAACC CCTCCATGAT CTCAGACCCA	180
	GACCAGCGAA CCGGAAGGGA GCAGCTGTCT AATGATGAGC TAATCAAGAA GTTAGTGACG	240
50	GAGCTGGCCG AGAATAGCAT GATCGAGGCT GAGGAGGTGC GGGGCACTCT TGGAGACATC	300

	TCGGCTCGTA TCGAGGCAGG GTTTGAGTCC CTGTCCGCCC TCCAAGTGA AACCATCCAG	360
	ACAGCTCAGC GGTGCGATCA CTCCGACAGC ATCAGGATCC TCGGCGAGAA CATCAAGATA	420
5	CTAGATCGCT CCATGAAGAC AATGATGGAG ACAATGAAGC TCATGATGGA GAAGGTGGAT	480
	CTCCTCTACG CATCAACCGC CGTTGGGACC TCTGCACCCA TGTTGCCCTC CCATCCTGCA	540
	CCTCCGCGCA TTTATCCCCA GCTCCCAAGT GCCCCGACAA CGGATGAGTG GGACATCATA	600
10	CCA	603

(2) INFORMATION FOR SEQ ID NO:5:

15 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 603 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

25 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

30	ATGGCAACGG AACCATCGAG TCTGGTCGAC TCCCTGGAGG ACGAAGAAGA TCCCCAGACA	60
	CTACGA <sup>1</sup> CGGG AACGATCGGG GTCACCAAGA CCACGGAAGG TCCCAAGGAA TGCATTGACC	120
35	CAAGCAGTAG ACCAGCTCCT GAAGGACCTC AGGAAGAACC CCTCCATGAT CTCAGACCCA	180
	GACCAGCGAA CCGGAAGGGA GCAGCTGTCTG AATGATGAGC TAATCAAGAA GTTAGTGACG	240
	GAGCTGGCCG AGAATAGCAT GATCGAGGCT GAGGAGGTGC GGGGCACTCT TGGAGACATC	300
40	TCGGCTCGTA TCGAGGCAGG GTTTGAGTCC CTGTCCGCCC TCCAAGTGA AACCATCCAG	360
	ACAGCTCAGC GGTGCGACCA CTCCGACAGC ATCAGGATCC TCGGCGAGAA CATCAAGATA	420
45	CTAGATCGCT CCATGAAGAC AATGATGGAG ACAATGAAGC TCATGATGGA GAAGGTGGAT	480
	CTCCTCTACG CATCAACCGC CGTTGGGACC TCTGCACCCA TGTTGCCCTC CCATCCTGCA	540
	CCTCCGCGCA TTTATCCCCA GCTCCCAAGT GCCCCGACAA CGGATGAGTG GGACATCATA	600
50	CCA	603

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 426 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATGAATTCAA AACATTCCTA TGTGGAGCTC AAGGACAAGG TAATCGTCCC TGGATGGCCC	60
ACACTGATGC TTGAGATAGA CTTTGTAGGG GGGACTTCAC GGAACCAGTT CCTTAACATC	120
CCATTCTTTT CAGTGAAAGA GCCTCTGCAG CTTCCACGCG AGAAGAAGTT GACCGACTAC	180
TTTACTATTG ACGTAGAACC AGCAGGTCAT TCCCTGGTCA ATATATACTT CCAGATTGAC	240
GACTTCTTGC TCCTAACACT CAACTCACTA TCTGTGTACA AGGACCCGAT TAGAAAATAC	300
ATGTTCTTAC GCCTCAACAA GGACCAGAGC AAGCACGCAA TCAATGCAGC CTTCAATGTC	360
TTTTCTTATC GGCTTCGGAA CATTGGTGTT GGTCTCTCTG GCCCGGACAT TCGATCTTCA	420
GGGCCT	426

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 426 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGAATTCAA AGCATTTCCTA TGTGGAGCTC AAGGACAAGG TAATCGTCCC TGGATGGCCC 60  
 ACACTGATGC TTGAGATAGA CTTTGTAGGG GGGACTTCAC GGAACCAGTT CCTTAACATC 120  
 5 CCATTTCCTT CAGTGAAAGA GCCTCTGCAG CTTCCACGCG AGAAGAAGTT GACCGACTAC 180  
 TTTACTATTG ACGTAGAACC AGCAGGTCAT TCCCTGGTCA ATATATACTT TCAGATTGAC 240  
 GACTTCTTGC TCCTAACACT CAACTCACTA TCTGTGTACA AGGACCCGAT TAGAAAATAC 300  
 10 ATGTTCTTAC GCCTCAACAA GGACCAGAGC AAGCACGCAA TCAATGCAGC CTTCAATGTC 360  
 TTTTCTTATC GGCTTCGGAA CATTGGTGTT GGTCTCTCTG GCCCGGACAT TCGATCTTCA 420  
 15 GGGCCT 426

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:  
 20 (A) LENGTH: 426 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

30 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

35 ATGAATTCAA *seq id claim 5.4* AGCATTTCCTA TGTGGAGCTC AAGGACAAGG TAATCGTCCC TGGATGGCCC 60  
 ACACTGATGC TTGAGATAGA CTTTGTAGGG GGGACTTCAC GGAACCAGTT CCTTAACATC 120  
 CCATTTCCTT CAGTGAAAGA GCCTCTGCAG CTTCCACGCG AGAAGAAGTT GACCGACTAC 180  
 40 TTTACTATTG ACGTAGAACC AGCAGGTCAT TCCCTGGTCA ATATATACTT CCAGATTGAC 240  
 GACTTCTTGC TCCTAACACT CAACTCACTA TCTGTGTACA AGGACCCGAT TAGAAAATAC 300  
 45 ATGTTCTTAC GCCTCAACAA GGACCAGAGC AAGCACGCAA TCAATGCAGC CTTCAATGTC 360  
 TTTTCTTATC GGCTTCGGAA CATTGGTGTT GGTCTCTCTG GCCCGGACAT TCGATCTTCA 420  
 GGGCCT 426

50 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 426 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGAATTCAA AGCATTCTTA TGTGGAGCTC AAGGACAAGG TAATCGTCCC TGGATGGCCC	60
ACACTGATGC TTGAGATAGG CTTTGTAGGG GGGACTTCAC GGAACCAGTT CCTTAACATC	120
CCATTTCTTT CAGTGAAAGA GCCTCTGCAG CTTCCACGCG AGAAGAAGTT GACCGACTAC	180
TTTACTATTG ACGTAGAACC AGCAGGTCAT TCCCTGGTCA ATATATACTT CCAGATTGAC	240
GACTTCTTGC TCCTAACACT CAACTCACTA TCTGTGTACA AGGACCCGAT TAGAAAATAC	300
ATGTTCTTAC GCCTCAACAA GGACCAGAGC AAGCACGCAA TCAATGCAGC CTTCAATGTC	360
TTTTCTTATC GGCTTCGGAA CATTGGTGTT GGTCTCTCTG GCCCGGACAT TCGATCTTCA	420
GGGCCT	426

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1521 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATGCAGCCTT CAATGTCTTT TCTTATCGGC TTCGGAACAT TGGTGTGGT CCTCTCGGCC	60
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	CGGACATTTCG ATCTTCAGGG CCTTAGCTGC AATACTGACT CCACTCCTGG ACTGATTGAC	120
	CTGGAGATAA GGCGACTTTG CCACACCCCA ACGGAAAATG TCATTTTCATG CGAGGTTAGT	180
5	TATCTCAACC ACACGACTAT TAGCCTCCCG GCAGTCCACA CATCATGCCT CAAGTACCAC	240
	TGCAAAACCT ATTGGGGATT CTTTGGTAGC TACAGCGCTG ACCGAATCAT AAATCGGTAC	300
10	ACTGGTACTG TTAAGGGTTG TCTAAACAAC TCAGCACCAG AGGACCCCTT CGAGTGCAAC	360
	TGGTTCTACT GCTGCTCGGC GATTACAACA GAGATCTGCC GATGCTCTAT TACAAATGTC	420
	ACGGTGGCTG TGCAAAACATT CCCACCGTTC ATGTACTGCA GTTTTGCAGA CTGCAGTACC	480
15	GTGAGCCAAC AGGAGCTAGA GAGTGGAAAG GCAATGCTGA GCGATGGCAG TACATTAACT	540
	TATACCCCGT ATATCCTACA GTCAGAAGTC GTGAACAAAA CCCTCAATGG GACCATACTC	600
	TGCAACTCAT CCTCTAAGAT AGTTTCCTTC GATGAATTTA GGCGTTCATA CTCCTAACG	660
20	AATGGTAGTT ACCAGAGCTC ATCAATCAAT GTGACGTGTG CAAACTACAC GTCGTCCTGC	720
	CGGCCCAGGT TGAAAAGGCG GCGTAGGGAC ACCCAGCAGA TTGAGTATCT AGTTCACAAG	780
25	CTTAGGCCCA CACTGAAAGA TGCATGGGAG GACTGTGAGA TCCTCCAGTC TCTGCTCCTA	840
	GGGGTGTTTG GTACTGGGAT CGCAAGTGCT TCTCAATTTT TGAGGAGCTG GCTCAACCAC	900
	CCTGACATCA TCGGGTATAT AGTTAATGGA GTTGGGGTTG TCTGGCAATG CCATCGTGTT	960
30	AATGTCACGT TCATGGCGTG GAATGAGTCC ACCTATTACC CTCCAGTAGA TTACAATGGG	1020
	CGGAAGTACT TCCTGAATGA TGAGGGAAGG TTACAAACAA ACACCCCCGA GGCAAGGCCA	1080
35	GGGCTTAAGC GGGTCATGTG GTTCGGCAGG TACTTCCTAG GGACAGTAGG GTCTGGGGTG	1140
	AAACCGAGGA GGATTCGGTA CAATAAGACC TCACATGACT ACCACCTGGA GGAGTTTGAG	1200
	GCAAGTCTCA ACATGACCCC TCAGACCAGT ATCGCCTCGG GTCATGAGAC AGACCGCATA	1260
40	AATCATGCCT ACGGAACGCA GGCTGATCTC CTTCCATACA CCAGGTCTAG TAATATAACA	1320
	TCTACGGATA CAGGCTCAGG CTGGGTGCAC ATCGGCCTAC CCTCATTTGC TTTCTCAAT	1380
45	CCCCTCGGGT GGCTCAGGGA CCTACTTGCA TGGGCAGCCT GGTGGGTGG GGTTCCTATAC	1440
	TTAATAAGTC TTTGTGTTTC CTTACCAGCC TCCTTCGCGA GGAGGAGACG CCTCGGCCGG	1500
50	TGGCAGGAAT AAACCGTACC G	1521

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1521 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATGCAGCCTT CAATGTCTTT TCTTATCGGC TTCGGAACAT TGGTGTTGGT CCTCTCGGCC	60
CGGACATTCG ATCTTCAGGG CCTTAGCTGC AATACTGACT CCACTCCTGG ACTGATTGAC	120
CTGGAGATAA GGCGACTTTG CCACACCCCA ACGGAAAATG TCATTTTCATG CGAGGTTAGT	180
TATCTCAACC ACACGACTAT TAGCCTCCCG GCAGTCCACA CATCATGCCT CAAGTACCAC	240
TGCAAAACCT ATTGGGGATT CTTTGGTAGC TACAGCGCTG ACCGAATCAT AAATCGGTAC	300
ACTGGTACTG TTAAGGGTTG TCTAAACAAC TCAGCACCAG AGGACCCCTT CGAGTGCAAC	360
TGTTTCTACT GCTGCTCGGC GATTACAACA GAGATCTGCC GATGCTCTAT TACAAATGTC	420
ACGGTGGCTG TGCAAAACATT CCCACCGTTC ATGTACTGCA GTTTTGCAGA CTGCAGTACC	480
GTGAGCCAAC AGGAGCTAGA GAGTGGAAAG GCAATGCTGA GCGATGGCAG TACATTAAC	540
TATACCCCGT ATATCCTACA GTCAGAAGTC GTGAACAAAA CCCTCAATGG GACCATACTC	600
TGCAACTCAT CCTCTAAGAT AGTTTCCTTC GATGAATTTA GCGGTTTATA CTCCCTAACG	660
AATGGTAGTT ACCAGAGCTC ATCAATCAAT GTGACGTGTG CAAACTACAC GTCGTCTCTG	720
CGGCCCAGGT TGAAAAGGCG GCGTAGGGAC ACCCAGCAGA TTGAGTATCT AGTTCACAAG	780
CTTAGGCCCA CACTGAAAGA TGCATGGGAG GACTGTGAGA TCCTCCAGTC TCTGCTCCTA	840
GGGGTGTTTG GTACTGGGAT CGCAAGTGCT TCTCAATTTT TGAGGAGCTG GCTCAACCAC	900
CCTGACATCA TCGGGTATAT AGTTAATGGA GTTGGGGTTG TCTGGCAATG CCATCGTGTT	960
AATGTCACGT TCATGACGTG GAATGAGTCC ACCTATTACC CTCCAGTAGA TTACAATGGG	1020
CGGAAGTACT TCCTGAATGA TGAGGGAAGG TTACAAACAA ACACCCCCGA GGCAAGGCCA	1080

	GGGCTTAAGC GGGTCATGTG GTTCGGCAGG TACTTCCTAG GGACAGTAGG GTCTGGGGTG	1140
	AAACCGAGGA GGATTCGGTA CAATAAGACC TCACATGACT ACCACCTGGA GGAGTTTGAG	1200
5	GCAAGTCTCA ACATGACCCC TCAGACCACT ATCACCTCGG GTCATGAGAC AGACCCCATATA	1260
	AATCATGCCT ACGGAACGCA GGCTGATCTC CTTCCATACA CCAGGTCTAG TAATATAACA	1320
	TCTACGGATA CAGGCTCAGG CTGGGTGCAC ATCGGCCTAC CCTCATTTGC TTTCCTCAAT	1380
10	CCCCTCGGGT GGCTCAGGGA CCTACTTGCA TGGGCAGCCT GGTGGGTGG GGTTCCTATAC	1440
	TTAATAAGTC TTTGTGTTTC CTTACCAGCC TCCTTCGCGA GGAGGAGACG CCTCGGCCCG	1500
15	TTGCAGGAAT AAACCGTACC G	1521

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1521 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

35	ATGCAGCCTT CAATGTCTTT TCTTATCGGC TTCGGAACAT TGGTGTGGT CCTCTCGGCC	60
	CGGACATTCTG ATCTTCAGGG CCTTAGCTGC AATACTGACT CCACTCCTGG ACTGATTGAC	120
	CTGGAGATAA GGCGACTTTG CCACACCCCA ACGGAAAATG TCATTTTCATG CGAGGTAGT	180
40	TATCTCAACC ACACGACTAT TAGCCTCCCG GCAGTCCACA CATCATGCCT CAAGTACCAC	240
	TGCAAAACCT ATTGGGGATT CTTTGGTAGC TACAGCGCTG ACCGAATCAT AAATCGGTAC	300
45	ACTGGTACTG TTAAGGGTTG TCTAAACAAC TCAGCACCAG AGGACCCCTT CGAGTGCAAC	360
	TGGTTCTACT GCTGCTCGGC GATTACAACA GAGATCTGCC GATGCTCTAT TACAAATGTC	420
	ACGGTGGCTG TGCAAACATT CCCACCGTTC ATGTACTGCA GTTTTGCAGA CTGCAGTACC	480
50	GTGAGCCAAC AGGAGCTAGA GAGTGGAAG GCAATGCTGA GCGATGGCAG TACATTAAC	540

TATACCCCGT ATATCCTACA GTCAGAAGTC GTGAACAAAA CCCTCAATGG GACCATACTC 600  
 TGCAACTCAT CCTCTAAGAT AGTTTCCTTC GATGAATTTA GCGGTTTATA CTCCCTAACG 660  
 5 AATGGTAGTT ACCAGAGCTC ATCAATCAAT GTGACGTGTG CAAACTACAC GTCGTCCTGC 720  
 CGGCCCAGGT TGAAAAGGCG GCGTAGGGAC ACCCAGCAGA TTGAGTATCT AGTTCACAAG 780  
 CTTAGGCCCA CACTGAAAGA TGCATGGGAG GACTGTGAGA TCCTCCAGTC TCTGCTCCTA 840  
 10 GGGGTGTTTG GTACTGGGAT CGCAAGTGCT TCTCAATTTT TGAGGAGCTG GCTCAACCAC 900  
 CCTGACATCA TCGGGTATAT AGTTAATGGA GTTGGGGTTG TCTGGCAATG CCATCGTGTT 960  
 15 AATGTCACGT TCATGACGTG GAATGAGTCC ACCTATTACC CTCCAGTAGA TTACAATGGG 1020  
 CGGAAGTACT TCCTGAATGA TGAGGGAAGG TTACAAACAA ACACCCCCGA GGCAAGGCCA 1080  
 GGGCTTAAGC GGGTCATGTG GTTCGGCAGG TACTTCCTAG GGACAGTAGG GTCTGGGGTG 1140  
 20 AAACCGAGGA GGATTCGGTA CAATAAGACC TCACATGACT ACCACCTGGA GGAGTTTGAG 1200  
 GCAAGTCTCA ACATGACCCC TCAGACCAGT ATCGCCTCGG GTCATGAGAC AGACCCCAT 1260  
 25 AATCATGCCT ACGGAACGCA GGCTGATCTC CTTCCATACA CCAGGTCTAG TAATATAACA 1320  
 TCTACGGATA CAGGCTCAGG CTGGGTGCAC ATCGGCCTAC CCTCATTTGC TTTCTCAAT 1380  
 CCCCTCGGGT GGCTCAGGGA CCTACTTGCA TGGGCAGCCT GGTGCGGTGG GGTCTATAC 1440  
 30 TTAATAAGTC TTTGTGTTTC CTTACCAGCC TCCTTCGCGA GGAGGAGACG CCTCGGCCGG 1500  
 TGGCAGGAAT AAACCGTACC G 1521

35 (2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 571 base pairs

(B) TYPE: nucleic acid

40 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

45 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

5 TTCAYACAGT AACGCCYAGC CTTGTGTTTC TATGTTTGCT AATCCCAGGA CTGCACGCTG 60  
 CGTTTGTTC ACGAGGGGTG CCTCGTGAAT CYTACCTGTC GACGCCTRTY ACGCGTGGRG 120  
 AACAGACTGT YGTTAAGACT GCRRAGTTTT ACGGGGAAAA GACRACRCAG CGTGATCTCA 180  
 CCGAGCTGGA GATCTCCTCT ATMTTCAGCC ATTGTTGCTC ATTACTAATW GGGGTTGTGA 240  
 TAGGATCGTC RTCTAAGATY AAAGCAGRAG CCGAGCAGAT CAAGAAAAGG TTTAAACTA 300  
 10 TGATGGCAGC CKTAAACCGG CCATCCCATG GTGAGACTGC TACACTACTY CAGATGTTTA 360  
 ATCCACATGA GGCTATAGAT TGGATTAACG GCCARCCCTG GGTAGGCTCC TTTGTGTTGY 420  
 15 CTCTACTAAC TACAGACTTT GAGTCCCCAG GTAAAGAATT YATGGAYCAG ATTAARCTTG 480  
 TCGCAAGTTA TGCRCAGATG ACTACGTACA CTACTATAAA GGAGTACCTC GCAGAATGYA 540  
 TGGATGCTAC CCTTACAATC CCYGTAGTTG C 571

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 571 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

40 TTCATACAGT AACGCCCAGC CTTGTGTTTC TATGTTTGCT AATCCCAGGA CTGCACGCTG 60  
 CGTTTGTTC ACGAGGGGTG CCTCGTGAAT CCTACCTGTC GACGCCTGTC ACGCGTGAG 120  
 AACAGACTGT TGTTAAGACT GCGAAGTTTT ACGGGGAAAA GACGACGCAG CGTGATCTCA 180  
 45 CCGAGCTGGA GATCTCCTCT ATCTTCAGCC ATTGTTGCTC ATTACTAATA GGGGTTGTGA 240  
 TAGGATCGTC GTCTAAGATC AAAGCAGGAG CCGAGCAGAT CAAGAAAAGG TTTAAACTA 300  
 TGATGGCAGC CTAAACCGG CCATCCCATG GTGAGACTGC TACACTACTC CAGATGTTTA 360  
 50 ATCCACATGA GGCTATAGAT TGGATTAACG GCCAACCTG GGTAGGCTCC TTTGTGTTGC 420

CTCTACTAAC TACAGACTTT GAGTCCCCAG GTAAAGAATT TATGGACCAG ATTAAGCTTG 480  
TCGCAAGTTA TGCACAGATG ACTACGTACA CTACTATAAA GGAGTACCTC GCAGAATGCA 540  
5 TGGATGCTAC CCTTACAATC CCTGTAGTTG C 571

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 571 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

20 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

25 TTCATACAGT AACGCCCAGC CTTGTGTTTC TATGTTTGCT AATCCCAGGA CTGCACGCTG 60  
CGTTTGTTCA CGGAGGGGTG CCTCGTGAAT CCTACCTGTC GACGCCTATC ACGCGTGGAG 120  
AACAGACTGT TGTTAAGACT GCGGAGTTTT ACGGGGAAAA GACGACGCAG CGTGATCTCA 180  
30 CCGAGCTGGA GATCTCCTCT ATCTTCAGCC ATTGTTGCTC ATTACTAATA GGGGTTGTGA 240  
TAGGATCGTC GTCTAAGATC AAAGCAGAAG CCGAGCAGAT CAAGAAAAGG TTAAAACTA 300  
35 TGATGGCAGC CGTAAACCGG CCATCCCATG GTGAGACTGC TACTACTC CAGATGTTTA 360  
ATCCACATGA GGCTATAGAT TGGATTAACG GCCAACCCTG GGTAGGCTCC TTTGTGTTGT 420  
CTCTACTAAC TACAGACTTT GAGTCCCCAG GTAAAGAATT TATGGACCAG ATTAAGCTTG 480  
40 TCGCAAGTTA TGCACAGATG ACTACGTACA CTACTATAAA GGAGTACCTC GCAGAATGCA 540  
TGGATGCTAC CCTTACAATC CCTGTAGTTG C 571

45 (2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- 50 (A) LENGTH: 571 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

5 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

10 TTCATACAGT AACGCCCAGC CTTGTGTTTC TATGTTTGCT AATCCCAGGA CTGCACGCTG 60  
 CGTTTGTTC ACGAGGGGTG CCTCGTGAAT CCTACCTGTC GACGCCTATC ACGCGTGGAG 120  
 15 AACAGACTGT TGTTAAGACT GCGAAGTTTT ACGGGGAAAA GACGACGCAG CGTGATCTCA 180  
 CCGAGCTGGA GATCTCCTCT ATCTTCAGCC ATTGTTGCTC ATTACTAATA GGGGTTGTGA 240  
 TAGGATCGTC GTCTAAGATC AAAGCAGGAG CCGAGCAGAT CAAGAAAAGG TTAAAACTA 300  
 20 TGATGGCAGC CTAAACCGG CCATCCCATG GTGAGACTGC TACACTACTC CAGATGTTTA 360  
 ATCCACATGA GGCTATAGAT TGGATTAACG GCCAACCTG GGTAGGCTCC TTTGTGTTGT 420  
 25 CTCTACTAAC TACAGACTTT GAGTCCCCAG GTAAAGAATT TATGGACCAG ATTAAGCTTG 480  
 TCGCAAGTTA TGCACAGATG ACTACGTACA CTACTATAAA GGAGTACCTC GCAGAATGCA 540  
 TGGATGCTAC CTTACAATC CCTGTAGTTG C 571

30

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 689 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

40

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

50 TGACCATGAG CTCAACGGCY CTAACYCAYC TTCTTAACCG GCTATCACAT ACTATCACTA 60  
 AGGGTGACTC CTTTGTTATT AACYTWGAYT ATAGYTCCTG GTGCAACGGT TTCCGACCAG 120

	AACTRCARGC CCCAMTCTGT CGTCAGTTGG ATCAGATGTT CAATTGCGGG TACTTCTTCA	180
	GGACTGGGTG CACACTGCCA TGCTTTACCA CGTTTATTAT TCARGACAGR TTCAACCCGC	240
5	CCTATTCCYT CMGTGGTGAG CCCGTTGAAG ACGGWGTYAC ATGCGCGGTT GGGACTAARA	300
	CAATGGGRGA GGGYATGAGG CAGAAACTAT GGACAATYCT TACGAGCTGC TGGGAGATAA	360
	TTGCTCTTCG GGAAATTAAC GTGACGTTTA AYATACTAGG CCARGGTGAT AATCAGACAA	420
10	TCATYRTACA TAAATCTGCA AGCCAAAATA AYCAGCTATT AGCGGAGCGA GCAYTRGGRG	480
	CYYTGTAACA GCATGCTAGA TTAGCTGGCC ATAACCTYAA GGTAGARGAA TGYTGGGTGT	540
15	CAGATTGTCT GTATGAGTAT GGAAAGAAGC TYTCTTCCG TGGTGTACCT GTCCCRGGCT	600
	GTTTGAAGCA GCTCTCRGG GTGACGGAYT CYACTGGRGA GYTATTCCCA AACCTATACT	660
	CAAAGTTAGC CTGCTWAACA TCATCRTGY	689

20

(2) INFORMATION FOR SEQ ID NO:18:

	(i) SEQUENCE CHARACTERISTICS:
25	(A) LENGTH: 689 base pairs
	(B) TYPE: nucleic acid
	(C) STRANDEDNESS: single
	(D) TOPOLOGY: linear

30	(ii) MOLECULE TYPE: cDNA
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	(iii) HYPOTHETICAL: NO
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35	(iv) ANTI-SENSE: NO
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

40	TGACCATGAG CTCAACGGCT CTAACCTACC TTCTTAACCG GCTATCACAT ACTATCACTA	60
	AGGGTGACTC CTTTGTTATT AACCTTGACT ATAGTTCCTG GTGCAACGGT TTCCGACCAG	120
	AACTGCAGGC CCCAATCTGT CGTCAGTTGG ATCAGATGTT CAATTGCGGG TACTTCTTCA	180
45	GGACTGGGTG CACACTGCCA TGCTTTACCA CGTTTATTAT TCAAGACAGG TTCAACCCGC	240
	CCTATTCCCT CAGTGGTGAG CCCGTTGAAG ACGGAGTTAC ATGCGCGGTT GGGACTAAAA	300
	CAATGGGGGA GGGCATGAGG CAGAAACTAT GGACAATCCT TACGAGCTGC TGGGAGATAA	360
50	TTGCTCTTCG GGAAATTAAC GTGACGTTTA ACATACTAGG CCAAGGTGAT AATCAGACAA	420



TCATCATACA TAAATCTGCA AGCCAAAATA ACCAGCTATT AGCGGAGCGA GCACTAGGGG 480  
 CCCTGTACAA GCATGCTAGA TTAGCTGGCC ATAACCTCAA GGTAGAGGAA TGCTGGGTGT 540  
 5 CAGATTGTCT GTATGAGTAT GGAAAGAAGC TTTTCTTCCG TGGTGTACCT GTCCCGGGCT 600  
 GTTTGAAGCA GCTCTCACGG GTGACGGATT CTACTGGAGA GCTATTCCCA AACCTATACT 660  
 CAAAGTTAGC CTGCTTAACA TCATCATGC 689

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 689 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TGACCATGAG CTCAACGGCT CTAATCACC TTCTTAACCG GCTATCACAT ACTATCACTA 60  
 30 AGGGTGACTC CTTTGTTATT AACCTTGAAT ATAGTTCTTG GTGCAACGGT TTCCGACCAG 120  
 AACTGCAGGC CCCAATCTGT CGTCAGTTGG ATCAGATGTT CAATTGCGGG TACTTCTTCA 180  
 35 GGAATGGGTG CAACTGCGA TGCTTTACCA CGTTTATTAT TCAAGACAGG TTCAACCCGC 240  
 CCTATTCCTT CAGTGGTGAG CCCGTTGAAG ACGGAGTTAC ATGCGCGGTT GGGACTAAAA 300  
 CAATGGGGGA GGGCATGAGG CAGAACTAT GGACAATCCT TACGAGCTGC TGGGAGATAA 360  
 40 TTGCTCTTCG GGAAATTAAC GTGACGTTTA ACATACTAGG CCAAGGTGAT AATCAGACAA 420  
 TCATCATACA TAAATCTGCA AGCCAAAATA ACCAGCTATT AGCGGAGCGA GCACTAGGGG 480  
 45 CCCTGTACAA GCATGCTAGA TTAGCTGGCC ATAACCTCAA GGTAGAGGAA TGCTGGGTGT 540  
 CAGATTGTCT GTATGAGTAT GGAAAGAAGC TTTTCTTCCG TGGTGTACCT GTCCCGGGCT 600  
 GTTTGAAGCA GCTCTCACGG GTGACGGATT CTACTGGAGA GCTATTCCCA AACCTATACT 660  
 50 CAAAGTTAGC CTGCTAAACA TCATCATGC 689

(2) INFORMATION FOR SEQ ID NO:20:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 201 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

15

Met Ala Thr Glu Pro Ser Ser Leu Val Asp Ser Leu Glu Asp Glu Glu  
1 5 10 15

20

Asp Pro Gln Thr Leu Arg Arg Glu Arg Ser Gly Ser Pro Arg Pro Arg  
20 25 30

Lys Val Pro Arg Asn Ala Leu Thr Gln Pro Val Asp Gln Leu Leu Lys  
35 40 45

25

Asp Leu Arg Lys Asn Pro Ser Met Ile Ser Asp Pro Asp Gln Arg Thr  
50 55 60

Gly Arg Glu Gln Leu Ser Asn Asp Glu Leu Ile Lys Lys Leu Val Thr  
65 70 75 80

30

Glu Leu Ala Glu Asn Ser Met Ile Glu Ala Glu Glu Val Arg Gly Thr  
85 90 95

35

Leu Gly Asp Ile Ser Ala Arg Ile Glu Ala Gly Phe Glu Ser Leu Ser  
100 105 110

Ala Leu Gln Val Glu Thr Ile Gln Thr Ala Gln Arg Cys Asp Tyr Ser  
115 120 125

40

Asp Ser Ile Arg Ile Leu Gly Glu Asn Ile Lys Ile Leu Asp Arg Ser  
130 135 140

45

Met Lys Thr Met Met Glu Thr Met Lys Leu Met Met Glu Lys Val Asp  
145 150 155 160

Leu Leu Tyr Ala Ser Thr Ala Val Gly Thr Ser Ala Pro Met Leu Pro  
165 170 175

50

Ser His Pro Ala Pro Pro Arg Ile Tyr Pro Gln Leu Pro Ser Ala Pro  
180 185 190

Thr Thr Asp Glu Trp Asp Ile Ile Pro  
195 200

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 201 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Ala Thr Gly Pro Ser Ser Leu Val Asp Ser Leu Glu Asp Glu Glu  
1 5 10 15

Asp Pro Gln Thr Leu Arg Arg Glu Arg Ser Gly Ser Pro Arg Pro Arg  
20 25 30

Lys Val Pro Arg Asn Ala Leu Thr Gln Pro Val Asp Gln Leu Leu Lys  
35 40 45

Asp Leu Arg Lys Asn Pro Ser Met Ile Ser Asp Pro Asp Gln Arg Thr  
50 55 60

Gly Arg Glu Gln Leu Ser Asn Asp Glu Leu Ile Lys Lys Leu Val Thr  
65 70 75 80

Glu Leu Ala Glu Asn Ser Met Ile Glu Ala Glu Glu Val Arg Gly Thr  
85 90 95

Leu Gly Asp Ile Ser Ala Arg Ile Glu Ala Gly Phe Glu Ser Leu Ser  
100 105 110

Ala Leu Gln Val Glu Thr Ile Gln Thr Ala Gln Arg Cys Asp His Ser  
115 120 125

Asp Ser Ile Arg Ile Leu Gly Glu Asn Ile Lys Ile Leu Asp Arg Ser  
130 135 140

Met Lys Thr Met Met Glu Thr Met Lys Leu Met Met Glu Lys Val Asp  
145 150 155 160

Leu Leu Tyr Ala Ser Thr Ala Val Gly Thr Ser Ala Pro Met Leu Pro  
165 170 175

Ser His Pro Ala Pro Pro Arg Ile Tyr Pro Gln Leu Pro Ser Ala Pro  
180 185 190

Thr Thr Asp Glu Trp Asp Ile Ile Pro  
195 200

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 201 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met	Ala	Thr	Glu	Pro	Ser	Ser	Leu	Val	Asp	Ser	Leu	Glu	Asp	Glu	Glu	1	5	10	15
Asp	Pro	Gln	Thr	Leu	Arg	Arg	Glu	Arg	Ser	Gly	Ser	Pro	Arg	Pro	Arg	20	25	30	
Lys	Val	Pro	Arg	Asn	Ala	Leu	Thr	Gln	Pro	Val	Asp	Gln	Leu	Leu	Lys	35	40	45	
Asp	Leu	Arg	Lys	Asn	Pro	Ser	Met	Ile	Ser	Asp	Pro	Asp	Gln	Arg	Thr	50	55	60	
Gly	Arg	Glu	Gln	Leu	Ser	Asn	Asp	Glu	Leu	Ile	Lys	Lys	Leu	Val	Thr	65	70	75	80
Glu	Leu	Ala	Glu	Asn	Ser	Met	Ile	Glu	Ala	Glu	Glu	Val	Arg	Gly	Thr	85	90	95	
Leu	Gly	Asp	Ile	Ser	Ala	Arg	Ile	Glu	Ala	Gly	Phe	Glu	Ser	Leu	Ser	100	105	110	
Ala	Leu	Gln	Val	Glu	Thr	Ile	Gln	Thr	Ala	Gln	Arg	Cys	Asp	His	Ser	115	120	125	
Asp	Ser	Ile	Arg	Ile	Leu	Gly	Glu	Asn	Ile	Lys	Ile	Leu	Asp	Arg	Ser	130	135	140	
Met	Lys	Thr	Met	Met	Glu	Thr	Met	Lys	Leu	Met	Met	Glu	Lys	Val	Asp	145	150	155	160
Leu	Leu	Tyr	Ala	Ser	Thr	Ala	Val	Gly	Thr	Ser	Ala	Pro	Met	Leu	Pro	165	170	175	
Ser	His	Pro	Ala	Pro	Pro	Arg	Ile	Tyr	Pro	Gln	Leu	Pro	Ser	Ala	Pro	180	185	190	

Thr Thr Asp Glu Trp Asp Ile Ile Pro  
195 200

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 142 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met Asn Ser Lys His Ser Tyr Val Glu Leu Lys Asp Lys Val Ile Val  
1 5 10 15

Pro Gly Trp Pro Thr Leu Met Leu Glu Ile Asp Phe Val Gly Gly Thr  
20 25 30

Ser Arg Asn Gln Phe Leu Asn Ile Pro Phe Leu Ser Val Lys Glu Pro  
35 40 45

Leu Gln Leu Pro Arg Glu Lys Lys Leu Thr Asp Tyr Phe Thr Ile Asp  
50 55 60

Val Glu Pro Ala Gly His Ser Leu Val Asn Ile Tyr Phe Gln Ile Asp  
65 70 75 80

Asp Phe Leu Leu Leu Thr Leu Asn Ser Leu Ser Val Tyr Lys Asp Pro  
85 90 95

Ile Arg Lys Tyr Met Phe Leu Arg Leu Asn Lys Asp Gln Ser Lys His  
100 105 110

Ala Ile Asn Ala Ala Phe Asn Val Phe Ser Tyr Arg Leu Arg Asn Ile  
115 120 125

Gly Val Gly Pro Leu Gly Pro Asp Ile Arg Ser Ser Gly Pro  
130 135 140

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 142 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

5 Met Asn Ser Lys His Ser Tyr Val Glu Leu Lys Asp Lys Val Ile Val  
1 5 10 15

Pro Gly Trp Pro Thr Leu Met Leu Glu Ile Asp Phe Val Gly Gly Thr  
20 25 30

10 Ser Arg Asn Gln Phe Leu Asn Ile Pro Phe Leu Ser Val Lys Glu Pro  
35 40 45

Leu Gln Leu Pro Arg Glu Lys Lys Leu Thr Asp Tyr Phe Thr Ile Asp  
50 55 60

15 Val Glu Pro Ala Gly His Ser Leu Val Asn Ile Tyr Phe Gln Ile Asp  
65 70 75 80

20 Asp Phe Leu Leu Leu Thr Leu Asn Ser Leu Ser Val Tyr Lys Asp Pro  
85 90 95

Ile Arg Lys Tyr Met Phe Leu Arg Leu Asn Lys Asp Gln Ser Lys His  
100 105 110

25 Ala Ile Asn Ala Ala Phe Asn Val Phe Ser Tyr Arg Leu Arg Asn Ile  
115 120 125

Gly Val Gly Pro Leu Gly Pro Asp Ile Arg Ser Ser Gly Pro  
130 135 140

30

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 142 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

45 Met Asn Ser Lys His Ser Tyr Val Glu Leu Lys Asp Lys Val Ile Val  
1 5 10 15

Pro Gly Trp Pro Thr Leu Met Leu Glu Ile Gly Phe Val Gly Gly Thr  
20 25 30

50 Ser Arg Asn Gln Phe Leu Asn Ile Pro Phe Leu Ser Val Lys Glu Pro

35

40

45

Leu Gln Leu Pro Arg Glu Lys Lys Leu Thr Asp Tyr Phe Thr Ile Asp  
50 55 60

5

Val Glu Pro Ala Gly His Ser Leu Val Asn Ile Tyr Phe Gln Ile Asp  
65 70 75 80

10

Asp Phe Leu Leu Leu Thr Leu Asn Ser Leu Ser Val Tyr Lys Asp Pro  
85 90 95

Ile Arg Lys Tyr Met Phe Leu Arg Leu Asn Lys Asp Gln Ser Lys His  
100 105 110

15

Ala Ile Asn Ala Ala Phe Asn Val Phe Ser Tyr Arg Leu Arg Asn Ile  
115 120 125

Gly Val Gly Pro Leu Gly Pro Asp Ile Arg Ser Ser Gly Pro  
130 135 140

20

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

25

(A) LENGTH: 503 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

35

Met Gln Pro Ser Met Ser Phe Leu Ile Gly Phe Gly Thr Leu Val Leu  
1 5 10 15

Val Leu Ser Ala Arg Thr Phe Asp Leu Gln Gly Leu Ser Cys Asn Thr  
20 25 30

40

Asp Ser Thr Pro Gly Leu Ile Asp Leu Glu Ile Arg Arg Leu Cys His  
35 40 45

Thr Pro Thr Glu Asn Val Ile Ser Cys Glu Val Ser Tyr Leu Asn His  
50 55 60

45

Thr Thr Ile Ser Leu Pro Ala Val His Thr Ser Cys Leu Lys Tyr His  
65 70 75 80

50

Cys Lys Thr Tyr Trp Gly Phe Phe Gly Ser Tyr Ser Ala Asp Arg Ile  
85 90 95

	Ile	Asn	Arg	Tyr	Thr	Gly	Thr	Val	Lys	Gly	Cys	Leu	Asn	Asn	Ser	Ala	
				100					105						110		
5	Pro	Glu	Asp	Pro	Phe	Glu	Cys	Asn	Trp	Phe	Tyr	Cys	Cys	Ser	Ala	Ile	
			115					120					125				
	Thr	Thr	Glu	Ile	Cys	Arg	Cys	Ser	Ile	Thr	Asn	Val	Thr	Val	Ala	Val	
			130					135				140					
10	Gln	Thr	Phe	Pro	Pro	Phe	Met	Tyr	Cys	Ser	Phe	Ala	Asp	Cys	Ser	Thr	
	145					150					155					160	
	Val	Ser	Gln	Gln	Glu	Leu	Glu	Ser	Gly	Lys	Ala	Met	Leu	Ser	Asp	Gly	
					165					170					175		
15	Ser	Thr	Leu	Thr	Tyr	Thr	Pro	Tyr	Ile	Leu	Gln	Ser	Glu	Val	Val	Asn	
				180					185					190			
	Lys	Thr	Leu	Asn	Gly	Thr	Ile	Leu	Cys	Asn	Ser	Ser	Ser	Lys	Ile	Val	
20			195					200					205				
	Ser	Phe	Asp	Glu	Phe	Arg	Arg	Ser	Tyr	Ser	Leu	Thr	Asn	Gly	Ser	Tyr	
		210					215					220					
25	Gln	Ser	Ser	Ser	Ile	Asn	Val	Thr	Cys	Ala	Asn	Tyr	Thr	Ser	Ser	Cys	
	225					230					235					240	
	Arg	Pro	Arg	Leu	Lys	Arg	Arg	Arg	Arg	Asp	Thr	Gln	Gln	Ile	Glu	Tyr	
				245						250					255		
30	Leu	Val	His	Lys	Leu	Arg	Pro	Thr	Leu	Lys	Asp	Ala	Trp	Glu	Asp	Cys	
				260					265					270			
	Glu	Ile	Leu	Gln	Ser	Leu	Leu	Leu	Gly	Val	Phe	Gly	Thr	Gly	Ile	Ala	
35			275					280					285				
	Ser	Ala	Ser	Gln	Phe	Leu	Arg	Ser	Trp	Leu	Asn	His	Pro	Asp	Ile	Ile	
		290					295				300						
40	Gly	Tyr	Ile	Val	Asn	Gly	Val	Gly	Val	Val	Trp	Gln	Cys	His	Arg	Val	
	305					310					315					320	
	Asn	Val	Thr	Phe	Met	Thr	Trp	Asn	Glu	Ser	Thr	Tyr	Tyr	Pro	Pro	Val	
					325					330				335			
45	Asp	Tyr	Asn	Gly	Arg	Lys	Tyr	Phe	Leu	Asn	Asp	Glu	Gly	Arg	Leu	Gln	
				340					345					350			
	Thr	Asn	Thr	Pro	Glu	Ala	Arg	Pro	Gly	Leu	Lys	Arg	Val	Met	Trp	Phe	
50			355					360					365				



	Gly	Arg	Tyr	Phe	Leu	Gly	Thr	Val	Gly	Ser	Gly	Val	Lys	Pro	Arg	Arg	
	370						375					380					
5	Ile	Arg	Tyr	Asn	Lys	Thr	Ser	His	Asp	Tyr	His	Leu	Glu	Glu	Phe	Glu	
	385					390					395					400	
	Ala	Ser	Leu	Asn	Met	Thr	Pro	Gln	Thr	Ser	Ile	Thr	Ser	Gly	His	Glu	
					405					410					415		
10	Thr	Asp	Pro	Ile	Asn	His	Ala	Tyr	Gly	Thr	Gln	Ala	Asp	Leu	Leu	Pro	
				420					425					430			
	Tyr	Thr	Arg	Ser	Ser	Asn	Ile	Thr	Ser	Thr	Asp	Thr	Gly	Ser	Gly	Trp	
			435					440					445				
15	Val	His	Ile	Gly	Leu	Pro	Ser	Phe	Ala	Phe	Leu	Asn	Pro	Leu	Gly	Trp	
		450					455					460					
20	Leu	Arg	Asp	Leu	Leu	Ala	Trp	Ala	Ala	Trp	Leu	Gly	Gly	Val	Leu	Tyr	
	465					470					475					480	
	Leu	Ile	Ser	Leu	Cys	Val	Ser	Leu	Pro	Ala	Ser	Phe	Ala	Arg	Arg	Arg	
					485					490					495		
25	Arg	Leu	Gly	Arg	Leu	Gln	Glu										
					500												

(2) INFORMATION FOR SEQ ID NO:27:

30 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 503 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

40	Met	Gln	Pro	Ser	Met	Ser	Phe	Leu	Ile	Gly	Phe	Gly	Thr	Leu	Val	Leu	
	1				5					10				15			
	Val	Leu	Ser	Ala	Arg	Thr	Phe	Asp	Leu	Gln	Gly	Leu	Ser	Cys	Asn	Thr	
45				20					25					30			
	Asp	Ser	Thr	Pro	Gly	Leu	Ile	Asp	Leu	Glu	Ile	Arg	Arg	Leu	Cys	His	
			35					40					45				
50	Thr	Pro	Thr	Glu	Asn	Val	Ile	Ser	Cys	Glu	Val	Ser	Tyr	Leu	Asn	His	
		50					55					60					

	Thr	Thr	Ile	Ser	Leu	Pro	Ala	Val	His	Thr	Ser	Cys	Leu	Lys	Tyr	His	65	70	75	80
5	Cys	Lys	Thr	Tyr	Trp	Gly	Phe	Phe	Gly	Ser	Tyr	Ser	Ala	Asp	Arg	Ile	85	90	95	
	Ile	Asn	Arg	Tyr	Thr	Gly	Thr	Val	Lys	Gly	Cys	Leu	Asn	Asn	Ser	Ala	100	105	110	
10	Pro	Glu	Asp	Pro	Phe	Glu	Cys	Asn	Trp	Phe	Tyr	Cys	Cys	Ser	Ala	Ile	115	120	125	
	Thr	Thr	Glu	Ile	Cys	Arg	Cys	Ser	Ile	Thr	Asn	Val	Thr	Val	Ala	Val	130	135	140	
15	Gln	Thr	Phe	Pro	Pro	Phe	Met	Tyr	Cys	Ser	Phe	Ala	Asp	Cys	Ser	Thr	145	150	155	160
	Val	Ser	Gln	Gln	Glu	Leu	Glu	Ser	Gly	Lys	Ala	Met	Leu	Ser	Asp	Gly	165	170	175	
20	Ser	Thr	Leu	Thr	Tyr	Thr	Pro	Tyr	Ile	Leu	Gln	Ser	Glu	Val	Val	Asn	180	185	190	
	Lys	Thr	Leu	Asn	Gly	Thr	Ile	Leu	Cys	Asn	Ser	Ser	Ser	Lys	Ile	Val	195	200	205	
	Ser	Phe	Asp	Glu	Phe	Arg	Arg	Ser	Tyr	Ser	Leu	Thr	Asn	Gly	Ser	Tyr	210	215	220	
30	Gln	Ser	Ser	Ser	Ile	Asn	Val	Thr	Cys	Ala	Asn	Tyr	Thr	Ser	Ser	Cys	225	230	235	240
	Arg	Pro	Arg	Leu	Lys	Arg	Arg	Arg	Arg	Asp	Thr	Gln	Gln	Ile	Glu	Tyr	245	250	255	
35	Leu	Val	His	Lys	Leu	Arg	Pro	Thr	Leu	Lys	Asp	Ala	Trp	Glu	Asp	Cys	260	265	270	
	Glu	Ile	Leu	Gln	Ser	Leu	Leu	Leu	Gly	Val	Phe	Gly	Thr	Gly	Ile	Ala	275	280	285	
40	Ser	Ala	Ser	Gln	Phe	Leu	Arg	Ser	Trp	Leu	Asn	His	Pro	Asp	Ile	Ile	290	295	300	
45	Gly	Tyr	Ile	Val	Asn	Gly	Val	Gly	Val	Val	Trp	Gln	Cys	His	Arg	Val	305	310	315	320
	Asn	Val	Thr	Phe	Met	Thr	Trp	Asn	Glu	Ser	Thr	Tyr	Tyr	Pro	Pro	Val	325	330	335	
50																				

Asp Tyr Asn Gly Arg Lys Tyr Phe Leu Asn Asp Glu Gly Arg Leu Gln  
340 345 350

5 Thr Asn Thr Pro Glu Ala Arg Pro Gly Leu Lys Arg Val Met Trp Phe  
355 360 365

Gly Arg Tyr Phe Leu Gly Thr Val Gly Ser Gly Val Lys Pro Arg Arg  
370 375 380

10 Ile Arg Tyr Asn Lys Thr Ser His Asp Tyr His Leu Glu Glu Phe Glu  
385 390 395 400

Ala Ser Leu Asn Met Thr Pro Gln Thr Ser Ile Ala Ser Gly His Glu  
405 410 415

15 Thr Asp Pro Ile Asn His Ala Tyr Gly Thr Gln Ala Asp Leu Leu Pro  
420 425 430

Tyr Thr Arg Ser Ser Asn Ile Thr Ser Thr Asp Thr Gly Ser Gly Trp  
435 440 445

20 Val His Ile Gly Leu Pro Ser Phe Ala Phe Leu Asn Pro Leu Gly Trp  
450 455 460

25 Leu Arg Asp Leu Leu Ala Trp Ala Ala Trp Leu Gly Gly Val Leu Tyr  
465 470 475 480

Leu Ile Ser Leu Cys Val Ser Leu Pro Ala Ser Phe Ala Arg Arg Arg  
485 490 495

30 Arg Leu Gly Arg Trp Gln Glu  
500

(2) INFORMATION FOR SEQ ID NO:28:

35

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 189 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: protein

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

His Thr Val Thr Pro Ser Leu Val Phe Leu Cys Leu Leu Ile Pro Gly  
1 5 10 15

50 Leu His Ala Ala Phe Val His Gly Gly Val Pro Arg Glu Ser Tyr Leu  
20 25 30

Ser Thr Pro Val Thr Arg Gly Glu Gln Thr Val Val Lys Thr Ala Lys  
 35 40 45  
 Phe Tyr Gly Glu Lys Thr Thr Gln Arg Asp Leu Thr Glu Leu Glu Ile  
 5 50 55 60  
 Ser Ser Ile Phe Ser His Cys Cys Ser Leu Leu Ile Gly Val Val Ile  
 65 70 75 80  
 Gly Ser Ser Ser Lys Ile Lys Ala Gly Ala Glu Gln Ile Lys Lys Arg  
 10 85 90 95  
 Phe Lys Thr Met Met Ala Ala Leu Asn Arg Pro Ser His Gly Glu Thr  
 100 105 110  
 15 Ala Thr Leu Leu Gln Met Phe Asn Pro His Glu Ala Ile Asp Trp Ile  
 115 120 125  
 Asn Gly Gln Pro Trp Val Gly Ser Phe Val Leu Pro Leu Leu Thr Thr  
 20 130 135 140  
 Asp Phe Glu Ser Pro Gly Lys Glu Phe Met Asp Gln Ile Lys Leu Val  
 145 150 155 160  
 25 Ala Ser Tyr Ala Gln Met Thr Thr Tyr Thr Thr Ile Lys Glu Tyr Leu  
 165 170 175  
 Ala Glu Cys Met Asp Ala Thr Leu Thr Ile Pro Val Val  
 180 185  
 30

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 189 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

45 His Thr Val Thr Pro Ser Leu Val Phe Leu Cys Leu Leu Ile Pro Gly  
 1 5 10 15  
 Leu His Ala Ala Phe Val His Gly Gly Val Pro Arg Glu Ser Tyr Leu  
 20 25 30  
 50 Ser Thr Pro Ile Thr Arg Gly Glu Gln Thr Val Val Lys Thr Ala Glu  
 35 40 45

Phe Tyr Gly Glu Lys Thr Thr Gln Arg Asp Leu Thr Glu Leu Glu Ile  
 50 55 60  
 5 Ser Ser Ile Phe Ser His Cys Cys Ser Leu Leu Ile Gly Val Val Ile  
 65 70 75 80  
 Gly Ser Ser Ser Lys Ile Lys Ala Glu Ala Glu Gln Ile Lys Lys Arg  
 85 90 95  
 10 Phe Lys Thr Met Met Ala Ala Val Asn Arg Pro Ser His Gly Glu Thr  
 100 105 110  
 Ala Thr Leu Leu Gln Met Phe Asn Pro His Glu Ala Ile Asp Trp Ile  
 115 120 125  
 15 Asn Gly Gln Pro Trp Val Gly Ser Phe Val Leu Ser Leu Leu Thr Thr  
 130 135 140  
 20 Asp Phe Glu Ser Pro Gly Lys Glu Phe Met Asp Gln Ile Lys Leu Val  
 145 150 155 160  
 Ala Ser Tyr Ala Gln Met Thr Thr Tyr Thr Thr Ile Lys Glu Tyr Leu  
 165 170 175  
 25 Ala Glu Cys Met Asp Ala Thr Leu Thr Ile Pro Val Val  
 180 185

(2) INFORMATION FOR SEQ ID NO:30:

30 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 189 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

40 His Thr Val Thr Pro Ser Leu Val Phe Leu Cys Leu Leu Ile Pro Gly  
 1 5 10 15  
 45 Leu His Ala Ala Phe Val His Gly Gly Val Pro Arg Glu Ser Tyr Leu  
 20 25 30  
 Ser Thr Pro Ile Thr Arg Gly Glu Gln Thr Val Val Lys Thr Ala Lys  
 35 40 45  
 50 Phe Tyr Gly Glu Lys Thr Thr Gln Arg Asp Leu Thr Glu Leu Glu Ile  
 50 55 60

Ser Ser Ile Phe Ser His Cys Cys Ser Leu Leu Ile Gly Val Val Ile  
 65 70 75 80  
 Gly Ser Ser Ser Lys Ile Lys Ala Gly Ala Glu Gln Ile Lys Lys Arg  
 5 85 90 95  
 Phe Lys Thr Met Met Ala Ala Leu Asn Arg Pro Ser His Gly Glu Thr  
 100 105 110  
 Ala Thr Leu Leu Gln Met Phe Asn Pro His Glu Ala Ile Asp Trp Ile  
 10 115 120 125  
 Asn Gly Gln Pro Trp Val Gly Ser Phe Val Leu Ser Leu Leu Thr Thr  
 130 135 140  
 15 Asp Phe Glu Ser Pro Gly Lys Glu Phe Met Asp Gln Ile Lys Leu Val  
 145 150 155 160  
 Ala Ser Tyr Ala Gln Met Thr Thr Tyr Thr Thr Ile Lys Glu Tyr Leu  
 20 165 170 175  
 Ala Glu Cys Met Asp Ala Thr Leu Thr Ile Pro Val Val  
 180 185  
 25 (2) INFORMATION FOR SEQ ID NO:31:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 229 amino acids  
 (B) TYPE: amino acid  
 30 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: protein  
 35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:  
 Thr Met Ser Ser Thr Ala Leu Thr His Leu Leu Asn Arg Leu Ser His  
 1 5 10 15  
 40 Thr Ile Thr Lys Gly Asp Ser Phe Val Ile Asn Leu Asp Tyr Ser Ser  
 20 25 30  
 Trp Cys Asn Gly Phe Arg Pro Glu Leu Gln Ala Pro Ile Cys Arg Gln  
 45 35 40 45  
 Leu Asp Gln Met Phe Asn Cys Gly Tyr Phe Phe Arg Thr Gly Cys Thr  
 50 55 60  
 50 Leu Pro Cys Phe Thr Thr Phe Ile Ile Gln Asp Arg Phe Asn Pro Pro  
 65 70 75 80

Tyr Ser Leu Ser Gly Glu Pro Val Glu Asp Gly Val Thr Cys Ala Val  
 85 90 95  
 Gly Thr Lys Thr Met Gly Glu Gly Met Arg Gln Lys Leu Trp Thr Ile  
 5 100 105 110  
 Leu Thr Ser Cys Trp Glu Ile Ile Ala Leu Arg Glu Ile Asn Val Thr  
 115 120 125  
 Phe Asn Ile Leu Gly Gln Gly Asp Asn Gln Thr Ile Ile Ile His Lys  
 10 130 135 140  
 Ser Ala Ser Gln Asn Asn Gln Leu Leu Ala Glu Arg Ala Leu Gly Ala  
 145 150 155 160  
 Leu Tyr Lys His Ala Arg Leu Ala Gly His Asn Leu Lys Val Glu Glu  
 15 165 170 175  
 Cys Trp Val Ser Asp Cys Leu Tyr Glu Tyr Gly Lys Lys Leu Phe Phe  
 20 180 185 190  
 Arg Gly Val Pro Val Pro Gly Cys Leu Lys Gln Leu Ser Arg Val Thr  
 195 200 205  
 Asp Ser Thr Gly Glu Leu Phe Pro Asn Leu Tyr Ser Lys Leu Ala Cys  
 25 210 215 220  
 Leu Thr Ser Ser Cys  
 225

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Met Ala Thr Gly Pro Ser Ser Leu Val Asp Ser Leu Glu Asp Glu Glu  
 1 5 10 15  
 Asp Pro

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Arg Ile Tyr Pro Gln Leu Pro Ser Ala Pro Thr Ala Asp Glu Trp Asp  
1 5 10 15

Ile Ile Pro

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Met Asn Ser Lys His Ser Tyr Val Glu Leu Lys Gly Lys Val Ile Val  
1 5 10 15

Pro Gly

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide



(v) FRAGMENT TYPE: internal

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Arg Leu Arg Asn Ile Gly Val Gly Pro Leu Gly Pro Asp Ile Arg Ser  
1 5 10 15

10 Ser Gly Pro

(2) INFORMATION FOR SEQ ID NO:36:

15 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Gly Leu Ser Cys Asn Thr Asp Ser Thr Pro Gly Leu Ile Asp Leu Glu  
1 5 10 15

30

Ile Arg

(2) INFORMATION FOR SEQ ID NO:37:

35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Arg Ser Lys Leu Arg Arg Arg Arg Arg Asp Thr Gln Gln Ile Glu Tyr  
1 5 10 15

50

Leu Val

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Leu Ile Ser Leu Cys Val Ser Leu Pro Ala Ser Phe Ala Arg Arg Arg  
1 5 10 15

Arg Leu Gly Arg Trp Gln Glu  
20

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Met Pro Pro Lys Arg Arg Leu Val Asp Asp Ala Asp Ala Met Glu Asp  
1 5 10 15

Gln Asp

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

10 Met Glu Asp Gln Asp Asp Leu Tyr Glu Pro Pro Ala Ser Leu Pro Lys  
1 5 10 15  
Leu Pro

15 (2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 amino acids

20 (B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

30 Glu Leu Ser Gly Glu Ile Ser Ala Ile Met Arg Met Ile Gly Val Thr  
1 5 10 15  
Gly Leu Val

35

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

40 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

45

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CAGGAGGCTC AATGGCAACG

20

5 (2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

15 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

TTTATGGTAT GATGTCCCAC

20

25 (2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

30 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

35 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

ATCGAATCAC CATGAATTCA AAGC

24

45 (2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

50 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

5 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

10

GTCAGTATTG CAACTAAGGC

20

(2) INFORMATION FOR SEQ ID NO:46:

15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

25 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

30

GCACGCAATT AATGCAGC

18

(2) INFORMATION FOR SEQ ID NO:47:

35 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

45 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

50

CAGTG TAGGC CTAAGCTTGT G

21

(2) INFORMATION FOR SEQ ID NO:48:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 20 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

AAGTTGAGAA GGCGGCGTAG

20

(2) INFORMATION FOR SEQ ID NO:49:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 19 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CGGTACGGTT TATTCCTGC

19

(2) INFORMATION FOR SEQ ID NO:50:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 19 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

5. (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

TGACCATGAG CTCAACGGC

19

(2) INFORMATION FOR SEQ ID NO:51:

10

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

20

(iv) ANTI-SENSE: NO

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GCATGATGAT GTTAAGCAGG C

21

(2) INFORMATION FOR SEQ ID NO:52:

30

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
35 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

40

(iv) ANTI-SENSE: NO

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

TTCATACAGT AACGCCCAGC

20

(2) INFORMATION FOR SEQ ID NO:53:

50

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

10

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

15

GCAACTACAG GGATTGTAAG GG

22

(2) INFORMATION FOR SEQ ID NO:54:

20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

30

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

35

GCCTTGTTGTT TCTATGTTTG C

21

(2) INFORMATION FOR SEQ ID NO:55:

40

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

50

(iv) ANTI-SENSE: NO



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

GCATCCATAC ATTCTGCGAG

20

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

CGAATTCGCA CGCAATTAAT GCAGC

25

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

GCTACTCGAG CGGTACGGTT TATTCCTGC

29

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

5 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

CCCCCGGGC AATGTACTGC AGTTTCGCGG ACT

33

15 (2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

20 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

25 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GGGCCCCGGGT TATTCCTGCC ACCGGCCGA

29

35 (2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

40 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

45 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

ATGCCACCCG GGAGACGCCT GATTGAT

27

(2) INFORMATION FOR SEQ ID NO:61:

- 5 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 32 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

15

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

20

CGGATCCCGG GCTAGTTTAG ACCAGTCACT CC

32

(2) INFORMATION FOR SEQ ID NO:62:

- 25 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 42 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

35

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

40

GGCCATATGC GCCCGGGCCC ATCGAGTCTG GTCGACTCCC TG

42

(2) INFORMATION FOR SEQ ID NO:63:

- 45 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 36 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

CTCGAGCCCG GGTATGGTA TGATGTCCCA CTCATC

36

10

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

15

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

20

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

CGAATCCCCG GGAATTCAAA GCATTCCTA

29

30

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

35

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

40

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

TCCCCCGGG CAGTATTGCA ACTAACGG

28

50